D2.22 By-products and waste from general industry for ECO-CEMENT production

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SUMMARY

Portland cement is a binder material and one of the most widely used materials in the construction industry, as it is cheap, diverse, with excellent material properties and can be adopted for many application processes and forms. Due to the extremely beneficial properties of concrete, attempts have been made to look for other cementation processes in the natural environment that can substitute Portland cement, such as the Biocementation. In fact, it is known that the phenomenon of Biocementation occurs widely in the natural environments over large time frames, for example in the formation of stalactites, stalagmites and sea shells. However, for industrial use this time frame has to be reduced and the rate of the reaction needs to be accelerated by the addition of suitable compounds to the process.

The ECO-CEMENT project is focused in one of the most promising alternatives to produce a Portland cement substitute: the "Microbial induced calcium carbonate precipitation (MICP)" by the use of ureolytic bacteria. These bacteria are widely available in the soil and natural environment, can be easily controlled and have the ability to produce cementation at a comparatively much faster rate.

The mechanism of reaction is as follows: the ureolytic bacteria hydrolyse urea to produce carbonate ions and in the presence of free calcium ions precipitate calcium carbonate. Urea is needed as primary reagent. If the saturation levels of the calcium carbonate produced are sufficiently high, it will precipitate forming bonds and consolidating its surroundings in the MICP process. As these processes use naturally existing components for the carbonate precipitation process, the environmental impacts of this material will not be as strong as Portland cement.

The rate at which the enzyme urease hydrolysis the substrate urea is called Urease Activity. There are bacteria that produce this enzyme in the cytoplasm of the cell for ATP generation. The urease enzyme is non-constitutive in nature, where its activity is dependent of urea and ammonia, and varies with the presence of calcium, pH, temperature and calcium nitrate.

To produce a high urease rate, the bacteria need a protein source to grow. Currently yeast extract is being used as a protein source: as it does not leave behind large particulate matter that could cause problems during cementation. However it is expensive. We also need a source of urea for the reaction to occur.

Hence, the purpose of the Deliverable 2.22 is to explore cheaper alternative protein sources that could be supplemented to the ureolytic bacteria to maintain high level production rates. Another purpose is to identify alternative sources of urea. These sources are relatively much cheaper than the laboratory industrial medium: however it is necessary to determine if they would require some preprocessing which would add a small additional cost in order to reduce the presence of contaminants and non desired bacteria. These facts will be explored in subsequent pages.

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1.- INTRODUCTION

Hydrolysis of urea is an irreversible reaction in which urea reacts with water to form ammonium and carbonate. The enzyme urease catalyzes the reaction significantly up to 1014 times faster. At neutral pH, bicarbonate (HCO_3^-) is the dominant carbonate species rather than carbonate (CO_3^{2-}), causing a rise in pH to maintain charge balance. As a result of the pH increment, ammonium (NH_4^+) starts to dissociate to ammonia (NH_3) until equilibrium is reached between NH_4^+/NH_3 and HCO_3^-/CO_3^{2-} at a pH of about 9.3. The produced carbonate ions precipitate in the presence of calcium ions and form calcium carbonate crystals, which form cementing bridges between the existing sand grains:

$$CO(NH_2)_2 + 2\,H_2O \xrightarrow{Urease} 2NH_4^+ + CO_3^{2-}$$

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3$$

Urease is a commonly found enzyme in many organisms, including many bacteria, some yeast and several higher plants. The specific urease activity differs from species to species. Jack Bean is the most common source of commercially available urease. The urease is available in different qualities varying from grinded bean meal to a purified powder, with specific activities ranging from 6 up to 1200 mol-urea L-1 min-1 gDW-1.

However, b*acterial cells are preferred over plants as a source of urease for biocementation* purposes. This is mainly as for biocementation with high strength to occur, nucleation sites are needed where CaCO3 crystal can grow and form bonds within sand grains. In plant sourced urease, the enzymes do not attach themselves to the sand particles, as the CaCO3 formed is not grown around the sand particle, but deposited in loosely packed layers. While in bacterial cells, they act themselves as nucleation sites, causing localized cementation at specific points that can be controlled: thus forming stronger bonds with the sand particles.

For the bacterial growth a suitable food source is needed. This will be assimilated by the bacteria and used as energy for its metabolism and reproduction. The protein source is used in the first phase of cultivation of the bacteria. Depending on the protein source used further processing of the bacteria may or may not be needed.

Traditionally, the protein source used in the biocementation experiments is yeast extract (YE), which is dissolved into the bacterial solution, leaving no particulate matter when consumed. The protein source needs to have high protein content and not contain particulate matter, harmful materials or residual matter which would harm the bacteria and reduce its urease activity. Based on these above assumptions the protein source will need more or less processing (more expensive).

The cost of well-defined media is prohibitively expensive for large scale cultivation of bacteria. The medium ingredients are a major cost factor, ranging between 10 to 60% of the total operating costs. Hence, large scale growth media is complex and waste or by-products from different industries should be identified to act as nutrient substitutes. There are many industrial effluents that are rich in proteins which if released in the altered form could be hazardous for the

atmosphere so, the dual benefits of cost reduction and environment protection are feasible by reusing these wastes.

Apart from the bacteria, urea is needed Urea is a major component used in the biocementation process. At present the pure chemical form is being used but cheaper alternatives need to be identified. Urea may be substituted for urine as it contains large concentrations of urea. However, even though sterile conditions are not needed for biocementation, it is necessary to study the effect of the presence of other chemicals in urine on the bacterial and cementation process.

Therefore, this Deliverable aims to identify suitable sources of inexpensive waste for the bacterial growth, and urea sources in order to develop an optimized medium prior to scale-up. The acceptance of these substitutes will be assessed as part of the work carried out in WP3.

The author wishes to stress that this Deliverable has a PUBLIC dissemination level. For that reason, the confidential information related to the research done within the project was preserved. The information displayed here was drawn from industrial practice, academic sources, such as journals, text books and also utilised web based research reports.

2.- PRELIMINARY REQUIREMENTS FOR THE INDUSTRIAL WASTE

Urease producing bacteria can be divided into two groups, according to their urease response to ammonium:

- Those, whose activities are repressed by high ammonium concentrations, for example *Pseudomonas areuginosa, Alcaligenes eutrophas, Bacillas megaterium* and *Klebsiella aerogenes*.
- And those whose activities are not repressed by ammonium. These are potentially suitable for biocementation (see the Table below):

Microorganism	High activity	Not repressed by NH_4^+	Not pathogenic
Sporosarcina Pasteurii	Yes	Yes	Yes
Proteus vulgaris	Unknown	Yes	Moderately
Proteus Mirabilis	Unknown	Yes	No
Helicobacter pyroli	Yes	Yes	No
Ureplasmas	Yes	Yes	No

Table 1 Microorganisms with urease activity that are not repressed in the presence of NH_4^+ [Whiffin, 2004]

From the Table above,, the most suitable microorganism will be selected in Task 3.1. The potential growth of the microorganism is dependent on the bacteria itself and the medium in which it is cultivated. Industrial by-products are said to be an alternative but the behavior of the bacteria in this media needs to be checked as part of WP3 tasks.

2.1 Bio-cementation process limitations

In the process of biocementation large quantities of urea are hydrolysed and as such for every molecule of urea assimilated two molecules ammonium are released. Thus the bacteria used have to be resistant to high concentrations of ammonium. The bio-cementation process has limitations, which should be considered within the selection process. The Bio-cementation process is a consequence of the metabolic process of some microorganisms. Microorganisms show a time dependent growth: while they are cultivated in complete media (see Fig. 1). Considering this growth curve, we compare the Log Phase entry of the microorganisms after contact with the 'potential industrial waste' in order to evaluate their (rest) vitality.

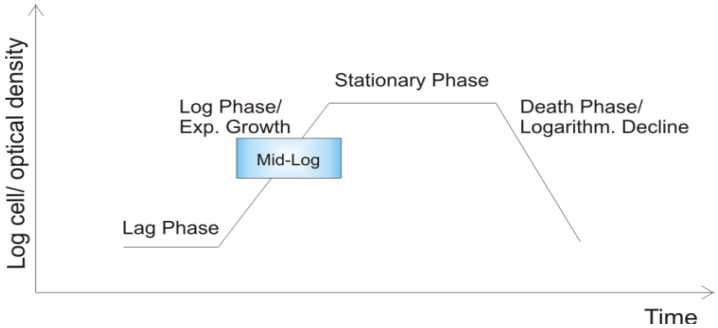

Figure 1 Growth curve of a fictitious microorganism.

The criterion for the upcoming actions for the selection of a suitable industrial waste is pointed out in the next section. An experiment to explore the compatibility of 'potential industrial waste' with the microorganism has been defined "the vitality test". The test measures the vitality of the microorganism after their incubation with the 'potential industrial waste'. This will be an instrument to evaluate the 'potential industrial waste' inter alia organic waste.

2.1.1 Favourable growth conditions for S. Pasteurii

Sporosarcina Pasteurii has been reported to be suitable for the biocementation process as the enzyme urease is present in the cytoplasm of this bacterial cell. Its favorable growth condition is 30°C. In order to provide them with oxygen, it needs to be shaken (50 rpm) and it needs to be feed with media (see Table 2).

20 g/L	peptides and aminoacids
20 g/L	Urea
5 g/L	Sodiumchloride (NaCl)
2,5 g/L	Dipotassiumphosphate (K_2HPO_4)
2,5 g/L	Glucose

Table 2 CASO Bouillon media

Taking into account that peptides, aminoacids and urea presents the compounds with the highest content (20 g/L), we will focus at first on the substitution of the peptides and aminoacids. Peptides are short chains of amino acid monomers linked by peptide (amide) bonds whereas one or more chains of Aminoacids form large biological molecules named Proteins.

The acceptance of substitutes in terms of feed for the microorganisms will be elaborated by the test method defined by IFAM "vitality test". In the next Table, the relevant types of stress and suggestions how to proceed in terms of evaluations are summarized.

Stressor	Stressor subtypes	Relevance for the cementation process
oxygen	Aerob	Low priority
	Anaerob	
medium	CASO	Medium priority
	Yeast	
proton	pH	Low priority
	T	Low priority
Ion	Type (e.g. Ca^{2+}, S^{2-})	High priority
	concentration	High priority
cells	Concentration (OD)	High priority
	Out of which growth state	High priority

Table 3 Classification for types of stress

A preliminary vitality test was conducted in order to check the acceptance of the microorganism to alternative sources of proteins. S. Pasteurii was feed with *Beer Yeast* instead of *CASO Bouillon*. The microorganisms survived, but their growth ratio was small. This suggests that additional experiments are needed varying the protein source and growing conditions. This will be deeply investigated in WP3.

3.- ALTERNATIVE SOURCES OF UREA AND NUTRIENTS

The medium ingredients in biotechnology processes are a major cost factor, ranging between 10 to 60% of the total operating costs. The medium cost increases proportionally with the size of the scale up. Because of this, it is important to give due consideration to optimization of the medium prior to scale up. Given that biocementation process does not require ease of removal of medium components or use of a defined medium, we are able to look at a range of more economical components to replace the existing expensive analytical grade chemicals.

3.1 Alternative sources of Urea

Urea is a colorless crystalline chemical compound, with formula $CO(NH_2)_2$, that is the major end product of protein metabolism in man and in other mammals. It can be found abundantly in the urine and faeces. In the industrial section, urea is used for many functional uses e.g. as adhesives, binders, sealants, resins, fillers, analytical reagents, catalysts, intermediates, solvent, dyestuffs, fragrances, deodourisers, flavouring agents, humectants and dehydrating agents, formulation components, monomers, paint and coating additives, photosensitive agents, fertilizers, surface treatment agents. Urea is also the key synthetic ingredient in the manufacture of some medicines. It is also widely used as an animal feed supplement. However, for the purpose of this report, commercial available forms will be discarded.

- Laboratory-grade Urea reference cost

Currently, the pure chemical form is being used at laboratory bur this may be substituted for other waste or industrial by-product. The cost of laboratory graded urea solution for microbiology, is *78.60€* per kg, (Sigma-Aldrich).

- Biocementation requirements

The amount required for biocementation was outlined by Al-Thawadi (2008). Al-Thawadi suggested that to obtain $1m^3$ of bioconcrete (20MPa) the ideal cementation solution concentration was 0.5M. This caused the precipitation of 145 mg of calcium carbonate per gram of aggregate. Assuming the above, he estimated that 340 kg of calcium carbonate were needed per m^3. Thus the urea needed was *204.19 kg* (urea molar weight = 60.06 $g.mol^{-1}$, calcium carbonate molar weight =100.87 $g.mol^{-1}$).

3.1.1 Urea in excretory products

Alternative sources of urea are the animal *manure i.e.: faeces and urine*. Urea is one of the major nitrogen excretory products of dairy cattle, sheep and many other large animals and hence, large animal operations located near waterways may be a source of urea. Non-ruminants animals are also a source of this nitrogen nutrient. Uric acid is the primary nitrogen form released by poultry, and the first decomposition product of uric acid is urea. The time scale of conversion from uric acid to urea depends on the microbial activity of the poultry litter and its moisture content.

The quantity of manure produced varies considerably among species because of differences in animal diets and metabolism and within species due primarily to differences in management. For

example, broiler litter may contain four to five times as much N, and ten times as much P, as horse manure.

Therefore, the nitrogen content in manure varies with the type of animal and feed ration, amount of litter, bedding or soil included, and amount of urine concentrated with the manure. Moisture content is also a major consideration. For example: The moisture content of fresh manure is around 70% to 85%. The moisture content of air-dried manure is around 9% to 15%. As manure dries, the nutrients not only concentrate on a weight basis, but also on a volume basis due to structural changes (settling) of the manure. Volatilization of urine nitrogen can result in considerable losses of nitrogen, up to 50% or more of the total amount used.

It can be estimated that about *half of the N in most animal manure is present in the soluble form as urea,* with the remaining half as insoluble organic compounds. The annual manure production estimates from various species is presented in the following table:

Animal type and size		Daily production	Approximate annual production				
Animal	Size	Manure	Manure	Nitrogen	Phosphate	Potash	Sulphur
Type	lb	lb/day	Tones/yr	lb N/year	lb P_2O_5/year	lb K_2O/year	lb S/year
Cattle							
Dairy cattle	500	43	7.8	25	25	60	5
	1,400	120	21.9	65	65	175	20
Beef cattle	750	45	8.2	35	40	65	5
	1,250	75	13.7	55	70	110	10
Swine							
Finishing pigs	150	9.8	1.8	5	5	15	5
	200	13.1	2.4	10	10	15	5
Sow and litter	375	22.5	4.1	15	10	30	5
Poultry							
Layers	4	0.21	0.038	0.50	0.50	0.35	0.05
Broilers	2	0.14	0.026	0.35	0.35	0.25	0.05

Table 4 Amount and Nutrient content of various types of manure (Source: Wisconsin Uni.)

In the manure, urine contains largest concentration of urea. Humans, like other mammals and amphibians, excrete liquid by-product urine with an average rate of 9.3 -18.6 gr/L of urea of per day. The average composition of human urine is presented below:

Compound	Chemical formula	Concentration
Urea	CO (NH2)2	9.3 g/L
Chloride	Cl^{-1}	1.87 g/L
Sodium	Na^{-1}	1.17 g/L
Potassium	K^{+}	0.750 g/L
Creatinine	$C_4H_7N_3O$	0.670 g/L
95% water, other ions, salts and organic matter		

Table 5 Human urine chemical compounds

Urine also contains pathogens: bacteria, viruses, parasitic protozoa and helminths though in low amounts; heavy metals: Cu, Zn, Cr, and Ni, Pb, Cd and hormones. Also, urine composition varies considerably among species because of differences in animal diets and metabolism.

"Published standard figures may not accurately reflect the urine composition to give the exact urea content. These suggest that the assessment for reusing urea has to be done in case by case basis".

Urine	Urea Content	Cost
Humans	9.3 g/L	Involves collection, transport and processing.
Cows	4.7 - 15 g/L	Involves collection, transport and processing.
Sheep	2.07-9.45 g/L	Involves collection, transport and processing.
Poultry	Uric acid content: 70% of total nitrogen content which ranges between 1.4-8.4% dry matter	Involves collection, transport and processing.

Urine	Availability	Processing	Determining factors
Humans	Although humans produce urine at a rate of 1-2 L per day, currently there are no means of collection of human urine as a solely product. Normally, it is disposed through as sewage sludge and processed in WWTP. Urine removal from TP is complex and	Processing is required to concentrate urea from urine (evaporation; dissolve in alcohol; and distillation is needed).	The composition and urine excretion in humans is not constant as it depends on environmental factors (humidity, T), weight, age, diet, etc.
Cows	6-12 L per day (average); 5-7 times per day	Processing is required to concentrate urea from urine	The composition is not constant as it depends on environmental factors (humidity, T), weight, age, diet, etc.
Sheep	0.5 - 1 L per day (average); 1-3 times per day	Processing required	The composition is not constant as it depends on environmental factors (humidity, T), weight, age, diet, etc.
Poultry	Hens (0.12 kg per day); chicken (0.08 kg/day); Turkey (0.3 kg/day)	Processing required	Depends on the specie, age, diet, health, environmental factors, etc.Collins, E.R., Barker, J.C., Carr, L.E., Brodie, H.L. y Martin, J.H. 1999. Poultry waste management handbook

- Human urine as an alternative urea source for biocementation: Literature review.

Urea is a major component used in biocementation. Urine could be an alternative urea source but, apart from Urea, it contains pathogens, metals, hormones, chemicals, etc. The presence of these substances in urine was studied with regards to its effects in the bacterial and cementation process. Van Paassen (2009) checked these effects and concluded that sodium chloride has similar effect on urease activity as calcium chloride but to a lesser extent. As such it could be regulated and controlled. However, the effects of potassium and other salts present were not investigated by him.

In a cementation solution with 0.5M urea, corresponding to 30.03g.L^{-1}, the total additional NaCl (58.44g.M^{-1}) was 9.9g.L-1, corresponding to 0.17moles. Thus the total inhibitory effect of salts present in the cementation solution was proportional to 0.67M (calcium chloride and sodium chloride). Preferential formation of calcium carbonate or sodium carbonate depended on the third law of thermodynamics. According to the equations below, calcium carbonate is the preferred compound as it will release more energy and there will be a greater drop in entropy.

Chemical equations

$$2NaCl + (NH_2)2CO + 2H_2O - > Na_2CO_3 + 2NH_4Cl$$
$$1130.77 + 2(407.27) + constant -> 316.23 + constant$$
$$CaCl_2 + (NH_2)2CO + 2H_2O -> Na_2CO_3 + 2NH_4Cl$$
$$1207.6 + 877.3 + constant -> 330.3 + constant$$

Compound	Standard enthalpy of formation	Energy released in reaction
NaCl	-411.12 KJ mol^{-1}	-316.23
Na_2CO_3	-1130.77	
$CaCl_2$	-877.3	-330.3
$CaCO_3$	-1027.6	

Table 6 Comparative standard entropy of formation of compounds formed by the addition of calcium chloride and sodium chloride and the exothermic energy released in kJ.mole-1 [Key Values for Thermodynamics, Hemisphere Publishing Corp., New York, 1989 Cox, J. D., Wagman, D. D., and Medvedev, V. A., CODATA]

Therefore, Van Passen determined that calcium carbonate was formed, if both sodium and calcium ions were present. Thus the process continued as long as there is sufficient supply of calcium chloride. However the formation of some sodium carbonate could not be ruled out and further study was needed.

3.1.2 Sewage sludge

In addition to the agriculture and the anthropogenic uses described above, there are other pathways by which urea reaches both the land and water environments. An important one is sewage, as urea is the major nitrogen component of urine. The extent to which this source of nitrogen is released to the environment depends on the state of the sewage treatment plant, the effectiveness of its mineralization and nitrification processes and the degree of nitrogen removal.

"The facts mentioned above make the supply inconstant and variable. For that reason, we have concluded that sewage sludge cannot be recommended as alternative urea source to produce ECO-CEMENT"

3.1.3 Conclusions

In the previous section available sources of inexpensive urea were identified. Traditional sources of urea included; excretory products (faeces and urine) and sewage sludge. Nonetheless, the typical generation values found in the literature were not representative. This suggests that an accurate assessment is needed in a case by case basis. For that, the following factors should be taken into account:

- Content of urea. The content of urea in the waste has to be sufficient to meet the biocementation requirements. Hence, an adequate characterization of the waste sample is the first step in the selection process.
- Cost. This involves the cost of the waste in origin, management, disposal, post processing and transport costs. Sources of inexpensive urea should be in the vicinity to avoid high hauling expenses and the associated GHG emissions. Comparison between the graded and the alternative urea cost is the reference fact that guides the selection of the urea source.
- Availability of the waste. Constant and reliable supply is needed for large scale operations.

The final decision will rely in a SWOT analysis matrix where the user can decide which is the convenient urea source, based on above listed facts.

Urea	Units	Laboratory-grade	Alternative Urea			
			Collection	Transport	Treatment	Total
Product Cost	€/kg	78.60				
Annual availability	ton/yr	Unrestricted	Depends on the animal specie, age, weight, diet and environmental factors. (Case by case analysis)			

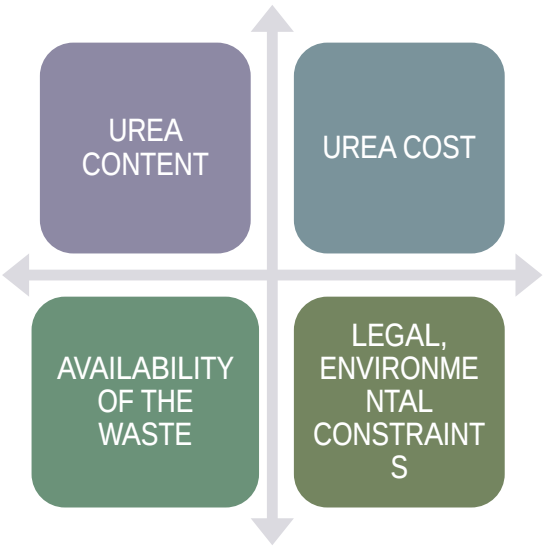

Figure 2 SWOT analysis matrix

3.2 Alternative sources of nutrients for the bacterial growth

For the large scale production of urease, it is necessary to find an inexpensive substrate for the bacteria to grow on that still produces a good level of urease activity. Low cost substrates are generally subject to lowered quality control and reproducibility. The effects of variable feedstock and additional processing required (e.g.: for the presence of insoluble particles when dissolved in water) should be considered in relation to the application of the enzyme produced.

We have found references in the literature that some of the protein component of the medium could be substituted by low cost alternative substrates such as: ethanol, acetate, glucose, citrate, fumarate, succinate and fructose. This has to be further investigated. However, if the consequences of applying this new media are likely to significantly reduce the efficiency of the process or application, it may be more economical to use a more expensive medium of reproducible composition.

Bearing all the above in mind, several alternative protein sources were identified to replace laboratory grade yeast extract.

Protein source	Description	Average Protein Content (%) Dry weight	Cost (per unit)
REFERENCE: Yeast extract	Water soluble portion of autolysed yeast. Supplied as powder. Completely soluble	66%	191 € per kg (Yeast Extract for microbiology. Sigma-Aldrich)
Corn Steep Liquor (CSL)	Concentrated aqueous liquid from steeping corn. Supplied as viscous liquid with suspended solid.	24%	82.20 €/kg (Sigma-Aldrich.) 3€ per liter (not processed).
Torula yeast (Candida utilis)	Supplied as a dried powder with suspended solids	45%	11.5 € per kg (NSA baits and feed. http://www.nsabaits.com/221-torula-yeast.html#)
Brewery waste yeast	Surplus yeast from brewing process. Whole non-lysed cells suspended in brewery waste.	48%	3 € per Kg (Cebadillo / bagazo. Different online suppliers)
Sludge biomass from WWTP	Concentrated sludge biomass from waste water treatment (WWTP) Whole heat killed non-lysed cells	25% (highly dependent on the process)	Free
Lactose Mother Liquor	High strength waste water generated by the lactose manufacturing milk plants.	8%	Free

Table 7 Alternative protein sources to replace laboratory grade yeast extract

3.2.1 Yeast extract and its derivative products

Yeast extract is the common name for various forms of processed yeast products made by removing the cell walls. They are used as food additives or flavorings, or as nutrients for bacterial culture media. Yeast extracts in liquid form can be dried to a light paste or a dry powder. Autolyzed yeast (containing the cell walls) or autolyzed yeast extract consists of concentrations of yeast cells that are allowed to die and break up, so that the yeasts' endogenous digestive enzymes break their proteins down into simpler compounds (amino acids and peptides).

Yeast autolysates are used in the following commercial products that are potential alternative substrates for bacterial growth:

Product	Country	Production/Supply	Cost
Vegetemite	Australia	Obtained from yeast extract as a by-product from brewing. Concentrated aytolysed yeast paste. Supplied as paste with suspended solids with a protein content of aprox. 40%	Prices range from 7.6 € per Kilo (Vegetemite. Kraft): 10€ per kilo (Marmite) to 70 € per kilo (Vitamin-R)
Marmite Promite Oxo	New Zealand: South Africa: United Kingdom: Republic of Ireland		
Cenovis	Switzerland		
Vitam-R	Germany		
Bovril	United Kingdom: Republic of Ireland		

Table 8 Yeast extract commercial derivative products

The general method for making yeast extract for food products such as Vegemite and Marmite on a commercial scale is to add sodium chloride (salt) to a suspension of yeast, making the solution hypertonic, which leads to the cells shriveling up: this triggers autolysis, in which the yeast self-destructs. The dying yeast cells are then heated to complete their breakdown, after which the husks (yeast with thick cell walls) are separated. Removing the cell walls concentrates the flavors and changes the texture. Yeast extract contains an amount of naturally occurring glutamic acid or monosodium glutamate: this is produced from an acid-base fermentation cycle, and is only found in some yeast, typically ones bred for use in baking.

We have found a reference in the literature to the use of Vegetemite for the production of urease activity compared to the laboratory-grade yeast extract. In that case, biomass concentration measurements were not fully reliably due to the presence of various concentrations of solids in the different media. However, it was concluded that high levels of urease activity and activity yield per gram were obtained with the application of both media (data not provided).

3.2.2 Corn Steep Liquor

Corn Steep Liquor is a viscous concentrate of corn solubles, rich in vitamins, amino acids, minerals and other growth stimulants. It contains approx. 50% (w/w) solids. Corn steep liquor is useful as an inexpensive alternative to peptone for a wide variety of microbiological production methods, including fed-batch production of recombinant proteins in *E. coli,* high density culture of *S. cerevisiae*, and fermentative production of lactic acid. The next Tables explain the average

composition of the major constituents of CSL and its physico-chemical characteristics (White and Johnson 2003).

CSL	
Component	Measure
pH	3.7 – 4.1
Solids (%)	45-50
Lactose (%)	5.8
Proteins (%)	24
Fats (%)	1
Ash (%)	8.8
Calcium (mg/l)	0.4
Phosphorus (mg/l)	0.5
Potassium (mg/l)	1.1
Sodium (mg/l)	0.9
Chloride (mg/l)	602
Sulphur (mg/l)	351

Table 9 CSL major constituents

Physical-Chemical Properties	Value
Form	Liquid
Colour	Tan to brown
Odour	Sharp
Melting Point	Not applicable
Boiling Point	100-101°C
Conductivity	2.5 µmoh
Specific Gravity	1.15-1.25
Density	1.2-1.4 g/cm3
Vapour Pressure	17.5 mm Hg at 25°C
Water Solubility	Soluble

Table 10 CSL Physico-chemical properties

Corn Steep Liquor has a pH of 3.7 to 4.1 and a specific gravity of 1.25. On the average 6.9% of the corn solids and 30% of the corn nitrogen are found in the steep liquor. CSL has a boiling point essentially that of water and the individual constituents of the mixture are highly water soluble nutrients. For this high solubility, little bioaccumulation can be found, though CSL does provide valuable nutrients that are naturally used by organisms for normal cellular subsistence and metabolism.

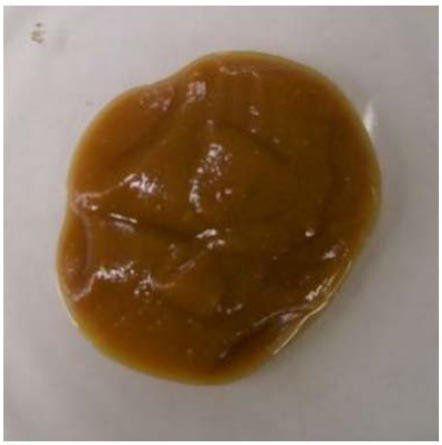

Figure 3 Sample of Corn Steep Liquor

The main disadvantage of corn steep liquor in microbiology is its variable composition. This variability may depend somewhat upon the type and condition of the corn but even more upon a multitude of variables in the processing of starch, but it is definitely an inexpensive alternative to yeast extract and peptone.

In laboratory, it can serve either as a supplement to replace extracts or as a main source of nitrogen and carbon for all microorganisms. In general, any organism capable of growing well on simple media containing beef extract and peptone will grow on media containing only corn steep liquor. If clear media is desired, preliminary treatment of the crude liquor becomes necessary. A good practice is to adjust the liquor with water until contains from 15 to 20% solids, raise the pH to 8 with potassium hydroxide concentrate, autoclave for an hour, and cool and filter. The filtrate then may be reconcentrated or refrigerated. Refrigeration of corn steep liquor is advisable to prevent spoilage by yeasts.

The following are some representative media: For yeasts, 0.5% corn steep liquor solids, with sugar as desired; for bacteria, 1.0% corn steep liquor solids, 0.5% glucose, adjusted to pH 7.4, as a basal medium. This can be modified in various ways, e.g., for the lactics, 1.0% corn steep liquor solids, 1.5% glucose in 1.0% phosphate buffer at pH 7.0. Also the following combinations have probed valuable after adjustment to the desired Ph: a, 0.5% corn steep liquor solids and 0.5% tryptone for bacteria: and b, 1.0% corn steep liquor solids and 0.3% yeast extract, for bacteria and yeasts.

- Microbial calcite precipitation with corn Steep Liquor. Literature review

Achal et al (2010) used this by-product for economization of the microbial calcite technology. Microbiological calcite precipitation was carried out in the CSL medium (1.5% v/v) and standard nutrient broth (NB). The chemical composition of the sample of CSL used consisted of 45–50% solids, 5.8% carbohydrates, 24% proteins, 1.0% fats, 8.8% minerals, and trace amounts of vitamins, and commercially available standard nutrient broth (NB) consisted of (g/l) casein hydrosylates 15.0, peptone 5.0, and NaCl 5.0 g/l. Corn steep liquor (CSL) was collected from the corn wet milling industry. Both media were supplemented with 2% urea and 25 mM CaCl2. The pH was adjusted to 6.5 with 1 N HCl prior to autoclaving without urea and CaCl2. Filter-sterilized urea and CaCl2 were added later.

Calcite production ability of S. Pasteurii (Bp M-3) was studied in NB and CSL media. One milliliter of overnight grown Bp M-3 culture was inoculated to 100 ml of NB and CSL media supplemented with 2% urea and 25 mM CaCl2. The bacteria were grown at 37ºC with continuous aeration at 120 rpm until their OD_{600} reached 1.0. The calcite precipitated in the medium was filtered through 0.45-lm membrane filter which also filters the bacterial cells. The calcite retained on the membrane was dried at 50ºC overnight and expressed as dry weight (mg)/cell dry mass (mg).

A microbial sand plugging was performed: 50 ml of S. Pasteurii (Bp M-3) culture (107 cells/ml) was mixed with 100 g sterilized river sand. The sand was sterilized prior to use in order to eliminate the indigenous microflora by autoclaving at 121ºC for 1 h, and this process was repeated three times at an interval of 24 h. Sand slurry containing bacterial culture was packed into a plastic column (height 15 in.: diameter 3 in.), and the bottom side of the column was blocked by using Whatman filter paper.

A control reaction was packed in a column in which sterilized sand was mixed with media alone (without cells). All columns were fed continuously with the corresponding media separately at room temperature to simulate the natural environmental conditions. Flow rate was measured by measuring the volume of media that came out of the columns per minute. The experiment was finished after 10 days and allowed to dry at room temperature. The sand columns were divided into three layers (upper, middle, and lower), and each layer was individually ground and sieved through a 45-lm-diameter mesh prior to calcite estimation. Precipitated calcite from each layer was measured by EDTA titration method.

Sporosarcina Pasteurii (Bp M-3) precipitated a significant level of calcite in both NB and CSL media. It produced 1.55 mg calcite/cell dry mass (mg) in CSL medium, while calcite production was 1.3 mg in NB medium. All sand columns prepared with S. Pasteurii (Bp M-3), using CSL and NB media were found to be tightly packed except the control sand column (after removing the plastic, it lost its form and collapsed). The flow rate of media was measured in the sand columns for 10 days. It was clearly observed that the flow rate changed over time as cementation and pore plugging progressed in the case of columns containing bacterial cells. The initial flow rate was recorded as 15.6 ml/min, while at the end of 10 days it was 13 ml/min in the case of control column. The flow was completely clogged at day 8 in the case of the column fed with CSL medium, while for NB medium complete clogging occurred at day 10 in bacteria-treated columns. All sand columns with bacterial cells were found to be tightly packed regardless of the media used.

The calcite content was measured at three levels of the column. It ranged between 33% at top level and 7% at the bottom. Calcite precipitation occurs predominantly in the areas close to the surface of the sand column. This is due to the fact that the facultative anaerobic bacteria cells like Bp M-3 grow at a higher rate in the presence of oxygen and consequently induce active precipitation of CaCO3 around the surface area While comparing the media, on calcite precipitation in sand, we found that the samples with CSL as nutrient source had marginally higher content of calcite compared with the standard nutrient broth. These results suggest that CSL can be used as an alternative nutrient source for biocalcification: such a replacement can reduce the process cost.

De Muynck et al. (2010) analysed the price of the microorganisms and the price of the nutrients. The calculated price of 1 kg lyophilized bacteria was about 1,100€. The cost of nutrients were estimated to be about 180 € per kg. For a successful commercial process the cost of nutrients is very high, but corn steep liquor can be available at a price of 3 € per liter aprox.., which is very economic compared to the standard nutrient medium.

In conclusion, the performance of CSL was significantly better than standard laboratory nutrients in terms of microbial concrete production. Also, CSL offers an economic advantage over the standard nutrient medium. Moreover, a potential environmental hazard is recycled beneficially. All these suggests that CSL can be used as an alternative nutrient source for biocalcification

3.2.3 Torula yeast (Candida Utilis)

Torula or Candida yeast refers to products containing Candida utilis, which have been used commercially for more than 60 years as nutritional supplements in animal feeds. Food grade Torula yeast is cultivated in mixtures of sugars and minerals, usually containing molasses, cellulosic wastes (e.g. spruce wood) or brewing by-products.

Figure 4 “Brown liquor” Sulfite waste liquor from paper industry: Fermentation leads to Torula yeast

The production of dried Torula yeast as waste product results from the fermentation of the waste sulfite liquors “brown liquor” from paper production. The yeast slurry, containing about 1% solids, is continuously separated from the substrate by centrifuging, the collected yeast cells are washed to removed all adhering chemicals and then dried to a moisture content of <7% to give the commercial product. This product has very high protein content (min 45%) and substantial amounts of B vitamins.

The process used by the chemical pulp industry today is the continuous growing of *Torula utilis* according to the Waldhof process. Whereas the baking yeast industry operates discontinuously in a batch operation with addition of both wort and chemicals and uses surface-active oily materials for foam control, the Waldhof process employs no such antifoams. Consequently, a very pure palatable yeast is produced which alone makes it possible to use the product for human consumption. Advantage is taken of the foaming tendency of waste liquor, first, to obtain intimate contact of the liquor plus its yeast content with the air, and secondly, to use as little air as possible and thus render the process more economical. While in batch operations 400 to 1,000 cubic feet of air per pound of yeast are required, the Waldhof method calls for only 150 cubic feet per pound or between one-third and one-sixth.

In the Waldhof method the sulfite waste liquor comes in neutralized and cooled down to 90°F. It flows continuously to the fermenter while at the same time ammonium, potash, magnesium, and phosphate are continuously added. The propagator is designed to disperse air mechanically into the liquid. Simultaneously, it forces vertical circulation of the liquid through the central draft tube. This prevents the accumulation of non-breaking foam on the surface. An aeration wheel acting as a centrifugal pump sucks in air and effects the conversion of the whole liquid into uniform air-mixed foam which it keeps in constant circulation.

After a retention time between three to five hours, depending on whether more hexose or more pentose sugars are added, the original yeast input has doubled itself and the sugars are practically consumed. Through a tap at the bottom of the fermenter the spent liquor with its high content of yeast is drawn off in quantities equal to the inflow of sugar-containing liquor at the top. A centrifugal device removes the air from the foamy liquor. The yeast is extracted by means of separators, washed several times and subsequently dried in towers or on drums.

In general terms, 8 to 10 tons of sulfite waste liquor is produced per ton of paper pulp. Sulfite waste liquor contains dissolved non pulp components of wood, mainly hemicelluloses, and the product of sulfonation of lignin—lignin sulfonic acid. Alcohol and nutrient yeast are obtained from sulfite waste liquor. Per each short ton of beech pulp produced, 200 to 240 lbs. (90 to 108 kg) of yeast can be grown.

The author has not found any literature reference to any previous experience. However, it should not be discarded as yeast substitute given its characteristics and nature.

3.2.4 Brewery waste yeast

Brewery waste yeast is surplus yeast from the brewing process. Low fermentation beer is produced through two fermentation steps: the primary fermentation being when 90% of the fermentable matter is consumed; the secondary fermentation and maturation, when a rapid cooling of the tank stops fermentation and causes flocculation of insoluble particles and the sedimentation of the yeast. The surplus yeast is recovered by natural sedimentation at the end of this second fermentation process. The tank bottom becomes full of yeast and “green beer”. This brewing by-product has dry matter content close to 10% w/w and generates beer losses of between 1.5 and 3% of the total volume of produced beer.

It is worth to recover at least a portion of the “green beer”, especially in countries where excessive taxes are paid on all beer produced in fermentation, including beer that is wasted. In order to be recovered, the “green beer” needs to be separated from the yeast. However, many breweries, especially smaller ones, discharge all surplus yeast without recovering any entrained beer contributing to considerable sewer loadings.

The amount of yeast grown depends on the fermentation conditions of each brewery, the type of yeast, the glycogen content, etc. Wort constituents that may affect yeast growth include carbohydrates, amino acids, free fatty acids, and trace minerals such as zinc.

Figure 5 Waste yeast in the tank bottom

The surplus yeast can be collected from the fermenter using one of the following methods:

- Pulling (ranking) settled yeast from the bottom of the fermenter.
- Separating yeast form the entire fermenter contents using feasing centrifuges.
- Pulling settled yeast followed by clarification of the rest of the fermenter contents in feasing centrifuges.
- Using various skimming systems when top-fermenting yeasts are employed.

Yeast cake obtained from presses or rotary vacuum filters can be discharged directly into bins for transport, broken up and bagged or pelletized, and stored in refrigerated containers. Larger breweries usually reslurry the yeast cake with water prior to further processing or sale. The reslurried yeast, as well as the yeast from centrifuges or yeast collected directly from fermenters or aging tank bottoms, is usually pumped to intermediate storage tanks where it awaits in-house processing or shipment to outside processors.

During storage, yeast can undergo changes that may not be desirable. It is important therefore to control storage and transportation conditions. Total solids may decrease when freshly harvested yeast slurry is stored depending on storage temperature. The decrease in solids during ambient storage may be accompanied by considerable foam formation. To prevent this, chilling of the yeast to below 5ºC is recommended. Agitators in the yeast storage tanks are useful to keep contents well mixed and prevent hot spots that accelerate metabolism. During fermentation, glycogen content peaks at about 50% of the cell mass: it may be still 30% when the yeast is harvested. As glycogen is metabolized during storage, ethanol and CO2 are formed. This CO2 may lead to excessive foaming. Another change during storage is that some yeast cells autolyze, releasing their cell constituents. This is why a considerable portion of yeast protein ends up in the liquid phase. The increase in protein concentration in the total slurry is merely a result of the decrease in carbohydrates.

Brewer´s yeast is considered as a source of a variety of biocatalysts, biochemical and metabolic intermediates. The most abundant element in yeast cells is carbon, which accounts for 50% of the dry weight. Other major elemental components are oxygen (30-35%), nitrogen (5%), hydrogen (5%) and phosphorous (1%).The most abundant classes of macromolecules are proteins and carbohydrates. The protein content present several bound amino acids, including

arginine, cystine, glycine, histidine, isoleucine, leucine, lysine, methonine, phenylalanine, theronine, tryptophan, tyrosine, and valine: leucine, lysine and tyrosine being the most abundant. It is also rich in vitamins (biotin, choline, folic acid, niacin, pantothenic acid, riboflavin, thiamin, and vitamin B-6), mainly niacin (Lewis and Young 1995: Huige 2006).

The total mineral content of yeast is approximately 5-10% of the cell dry weight. This fraction comprises a multitude of elements, especially potassium and phosphorous. The composition of some of them is shown in the following Table.

Components (% dry weight basis)	Reference (Huige 2006)	Reference (Lamoolphak 2006)	Reference (Lewis and Young 1995)
Protein	48	nr	50
Lipid	nr	1	nr
Ash	7	8	7
Crude fiber	3	nr	nr
Carbohydrates	nr	36	42
Minerals in ash (%)			
Calcium	0.12	nr	nr
Chlorine	0.12	nr	nr
Iron	0.01	nr	nr
Magnesium	0.24	nr	nr
Phosphorous	1.43	nr	nr
Potassium	1.71	nr	nr
Sodium	0.09	nr	nr
Sulfur	0.38	nr	nr
Vitamins (mg/100 g)			
Niacin	nr	nr	50
Thiamin	nr	nr	15
Panthotenate	nr	nr	10
Riboflavin	nr	nr	7
Folic acid	nr	nr	4
Pyridoxine	nr	nr	3
Biotin	nr	nr	0.2

Table 11 Chemical composition of surplus yeast

This surplus yeast is sold by many brewers at an average cost of 3€ per kilo, mainly to the animal feed industry. Payments are based on the weight of total solids of the yeast slurry as delivered.

Enzymes, proteins, vitamins, amino acids among others can be isolated from brewery´s yeast (Huige 2006). Protein and amino acids, for example, can be recovered by employing various processes such as autolysis, plasmolysis in organic salt solution or non-polar organic solvent, acid or alkali catalyzed hydrolysis, enzymatic hydrolysis, or hydrothermal decomposition (Lamoolphak et al. 2006).

- Microbial calcite precipitation with Brewery waste yeast. Literature review

Due to the composition rich in protein, amino acids, minerals, and other compounds of interest, several attempts have been done aiming to reuse the surplus yeast in biotechnological processes as source of nutrients. We have found a reference in the literature to the use of brewery waste yeast for the production of urease activity. Brewery waste yeast is largely comprised of whole cells, which resulted inaccessible to the growing microorganisms. Despite of that, initial attempts to lyse the cells were made by exposing them to 0.5 M NaOH for 20 minutes, followed by addition of H_2SO_4 to neutralise the cell extract to pH 8 (Schutte and Kula, 1990). However, this procedure produced an extract that did not sustain growth of *S. Pasteurii* (data not shown) so further investigation is required.

3.2.5 Lactose mother liquor (LML)

Lactose mother liquor is very high strength waste water generated by the lactose manufacturing milk plants. It is the residual liquor left behind after recovery of lactose from concentrated whey permeates. Due to its high strength, it is not easily amenable for treatment to meet the prescribed effluent standards. Every 10 liters of raw milk processed in the milk plant to produce lactose, generates 1 liter of lactose mother liquor. LML has high residual lactose (up to 15% or more), whey proteins (up to 8% or more) and milk minerals and the salts (as high as 7%) hence it cannot be viewed as waste water. Due to high lactose content (15% or more), LML can be used as a base culture medium for the production of value added products using biochemical conversion process.

Figure 6 Lactose Mother Liquor

- Microbial calcite precipitation with Lactose Mother Liquor. Literature review

Achal et al (2009) investigated the effect of LML as sole source of growing bacterium *S. Pasteurii* and compared the calcification effect of its usage. LML served as a better nutrient source for the growth of bacteria and also for calcite precipitation as compared to nutrient broth and yeast extract media which are quite expensive.

Microbiologically induced calcite precipitation by the bacterium *Sporosarcina Pasteurii* (NCIM 2477) using the industrial effluent of the dairy industry, lactose mother liquor (LML) as growth medium was demonstrated for the first time for Achal et al (2009). The urease activity and the calcite precipitation by the bacterium was tested in LML and compared with the standard media like nutrient media and yeast extract media. Calcite constituted 24.0% of the total weight of the sand samples plugged by S. Pasteurii and urease production was found to be 353 U/ml in LML medium. The compressive strength of cement mortar was increased by S. Pasteurii in all the media used compared to control. No significant difference in the growth, urease production and

compressive strength of mortar among the media suggesting LML as an alternative source for standard media.

Lactose mother liquor (LML), an industrial effluent of the dairy industry is used as the sole source of nutrients to grow the bacterium Sporosarcina Pasteurii (B. Pasteurii NCIM 2477). Its efficiency with other standard media has been compared. Studies were also performed to evaluate microbiological calcite precipitation, urease production and compressive strength of mortar in LML and compared with the standard growth media.

- Materials and methods

Microorganism and media: Sporosarcina Pasteurii NCIM 2477 was used. The culture was routinely maintained on Nutrient agar (pH 8.0) medium. Lactose mother liquor was collected from the dairy industry and analysed for its physicochemical properties (Table 12).

LML	
Component	Measure
pH	6.2
Solids (%)	5.5
Lactose (%)	15.4
Proteins (%)	8
Fats (%)	2
Ash (%)	0.53
Calcium (mg/l)	353
Phosphorus (mg/l)	35
Potassium (mg/l)	186
Sodium (mg/l)	44
Chloride (mg/l)	90
Sulphur (mg/l)	15

Table 12 Physico- chemical characteristics of lactose mother liquor (LML).

Microbiological urease production, calcite precipitation and compressive strength tests were carried out in the following three media (per liter): LML-urea medium (10% LML, 5 g NaCl, 2% urea and 25 mM CaCl2), NB-urea medium [8 g nutrient broth, 5 g NaCl, 2% urea and 25 mM CaCl2] and YE-urea medium [1 g yeast extract, 5 g NaCl, 2% urea and 25 mM CaCl2]. The pH of the media was adjusted to 6.5 with 1 N HCl prior to autoclaving without urea and CaCl2. Filter-sterilized urea and CaCl2 was added later.

The growth profile of S. Pasteurii in three media was tested by taking the absorbance (OD_{600}) at regular time intervals and corresponding cfu/ml were counted after overnight incubation at 37ºC.

Urease activity: The urease activity was determined in all three media according to the phenol–hypochlorite assay method. Ammonium chloride (50–1,000 μM) was used as the standard. The culture filtrates (250 μl) were added to a mixture containing 1 ml of 0.1 M potassium phosphate buffer (pH 8.0) and 2.5 ml of urea (0.1 M). The mixture was incubated at 37ºC for 5 min followed by addition of phenol nitroprusside and alkaline hypochlorite, 1 ml each and incubated at 37ºC for

25 min. Optical density was measured at 626 nm and one unit of urease is defined as the amount of enzyme hydrolyzing 1 μmole urea/min.

Microbiological sand plugging: Microbiological sand plugging was performed to study calcite precipitation. Fifty milliliters of grown culture (OD_{600} = 1.0) was mixed with 100 g sterilized river sand and was packed into a plastic column (height = 15 in.: diameter = 3 in.) and bottom side of column was blocked using Whatman filter paper. A control reaction was packed in column in which sterile sand was mixed with different media only (without bacteria). All columns were fed continuously with three specific media separately at room temperature to mimic the natural environmental conditions. The experiments in all the sand columns were terminated after 10 days. Microbial sand column was divided into three layers (upper, middle and lower layer) and each layer was individually ground and sieved through a 45 μm diameter mesh prior to calcite estimation. Precipitated calcite from each layer was measured by EDTA titration method. One gram of sand sample from each layer was dissolved with 3 N HCl and 4 ml of 5 N NaOH was added to the precipitate and the final volume was made up to 50 ml using distilled water. Few drops of hydroxyl napthol blue were added as an indicator and the mixture was finally titrated against 0.05 M EDTA. End point was noted from pink to blue, and the amount of CaCO3 formed was calculated by the volume of EDTA used x 0.005004 x 1,000/ml of sample used.

Compressive strength test: Locally available clean, dry, well graded, natural river sand was mixed with cement (3:1 w/w). A cube mold of 70.6 mm was prepared, as per IS 4031–1988. Sand and cement were thoroughly mixed, and added along with the grown culture of *S. Pasteurii* correspondence to OD_{600} of 1.0. Cubes were cast and compacted in a vibration machine. After de-molding all specimens were cured in corresponding medium at room temperature until compression testing at the intervals of 3, 7 and 28 days. Compression testing was performed using automatic compression testing machine, COMPTEST 3000. All the experiments were performed in triplicate. The data was analysed by Analysis of Variance (ANOVA) and the means were compared using Tukey's test. All the analyses were performed using GraphPad Prism (4.1) software.

- Results and discussion

Growth and pH profile: The growth profile studies up to 30 h in different media showed that *S. Pasteurii* has the ability to grow well and utilize the nutrients in LML along with other standard media. The growth profile was similar in all media and there was no significant difference observed among the media in relation to their cfu/ml. The pH of the medium significantly increased as the growth increased in all the media. The maximum pH of 11.0 was observed after 30 h of incubation. The pH profile did not differ significantly among the media. Some bacterial species may be able to use more complex biological polymers such as lipids, polysaccharides and nucleic acids because these organisms produce a range of extracellular hydrolytic enzymes that can degrade large organic molecules. The growth of *S. Pasteurii* appeared to be correlated with the pH. The rise in pH causes a precipitation of calcium carbonate, which occurs during urea degradation. In medium containing urea and CaCl2, that supports microbial growth, $NH4^+$ and Cl^- react with OH^- and H^+.

Urease activity: *Sporosarcina Pasteurii* showed maximum urease production in NB-urea medium (412 U/ml) followed by YE-urea medium (366 U/ml) and LML-urea medium (353 U/ml). The

highest productivities in all the media were obtained at 120 h. Urease production in NB-urea medium and YE-urea medium was 0.17- and 0.04 fold higher than those in LML-urea medium, which was not statistically significant. After 120 h, urease production was decreased in all the media. Bacteria are known to hydrolyze urea through urease for the purposes of: (1) increasing the ambient pH, (2) utilizing it as a nitrogen source, and (3) using it as a source of energy. In biological systems, many calcareous organisms couple calcification to their metabolic assimilation processes to scavenge protons. The subsequent increase of pH in surrounding medium due to the presence of ammonia ions and the additional release of CO2 from the enzymatic urea hydrolysis further accelerate the rate of the urease induced calcite precipitation. Thus, an active participation of urease is of essence in biochemical calcite precipitation.

Calcite precipitation in sand plugging:

Bacillus species are known to produce a large amount of urease in soil environments. The urease identified from these bacteria is found to be extracellular, so it can be directly applied to consolidate the sand column for calcite precipitation rather than the whole bacterial cells. All sand columns prepared with *S. Pasteurii*, using three media were found to be tightly packed except control sand column (after removing the plastic, it lost its form and collapsed). Calcite content was found to be maximum in case of upper layer of microbial sand column prepared with all the three media, as compared to the other two layers. Calcite constituted 28.4, 26.3 and 24.0% of the total weight of the sand samples plugged by S. Pasteurii in NB-urea medium, YE-urea medium and LML-urea medium, respectively. Calcite precipitation occurred predominantly in the areas close to the surface of the sand column. It is mainly due to the fact that facultative anaerobic *S. Pasteurii* grows at a higher rate in the presence of oxygen and consequently induces active precipitation of CaCO3 around the surface area. There was not much significant difference in calcite content produced by S. Pasteurii grown in LML medium as compared to other media. LML provides variety of ions in the medium in the form of Ca, Na, K, Mg and other elements. Due to which bacterial cell surface could nonspecifically induce mineral deposition by providing a nucleation site. Especially, Ca2+ is not likely to be utilized by microbial metabolic processes: it rather accumulates outside the cell. Possible biochemical reactions in medium containing urea and CaCl2 to precipitate CaCO3 at the cell surface can be summarized as follows:

Ca2+ + Cell -> Cell - Ca2+
Cl- + HCO3- + NH3 -> NH4Cl + CO_3^{2-}
Cell-Ca^{2+} + CO_3^{2-} -> Cell-CaCO3

Muynck et al. indicated that the type of bacterial culture and media composition have a profound impact on calcite crystal morphology. Crystal growth can be inhibited or altered by the adsorption of proteins, organic matter or inorganic components to specify crystallographic planes of the growing crystals. Differences in size and morphology between the different types of calcium source and different types of bacterial culture can also be attributed to the presence of organic matter. Hammes et al. suggested that differences in crystal morphology, which are obtained with different bacterial cultures, could be due to the level of the actual urease activity. In this study, the CaCO3 precipitation and urease activity was similar in all the three media tested with S. Pasteurii. This indicates that LML can serve as an alternative medium for precipitation of calcite in place of nutrient medium or yeast medium, which is costlier. In addition, LML offers a greener option by recycling industrial effluent.

Compressive strength: The compressive strength had increased for the mortar cubes that contained microbial cells irrespective of the media used to grow the cells compared to control. The highest compressive strength was obtained with mortar cubes that were incubated for 28 days. Mixing of S. Pasteurii in mortar cubes with LML-urea medium showed around 17% improvement in compressive strength at 28 days (26.3 MPa) with respect to control (23.2 MPa): whereas in case of NB-urea and YE-urea media, it was 27.9 and 27.2 MPa, respectively.

However, there was no significant difference observed in compressive strength among the media. This improvement in compressive strength is probably due to deposition of CaCO3 on the bacterial cell surfaces and within the pores of cement–sand matrix, which plug the pores within the mortar. The compressive strength was similar in all the treatments at 3 and 7 days of curing. Ghosh et al. also showed the improvement of compressive strength of cement mortar by the addition of Shewanella species. The overall trend of an increase in compressive strength up to 28 days might be attributed to the behavior of microbial cells within the cement mortar matrix. During the initial curing period, microbial cells obtained good nourishment, because the cement mortar was still porous: but growth might not be proper due to the completely new environment for microbes. It may also be possible that due to the cement pH remaining high, cells were in an inactive condition and while the curing period was increased, they started growing slowly. Upon cell growth, calcite would have precipitated on the cell surface as well as within the cement mortar matrix. Once many of the pores in the matrix were plugged, the flow of the nutrients and oxygen to the bacterial cells stopped, eventually the cells either died or turned into endospores and acted as an organic fiber, increasing the compressive strength of the mortar cubes. This explains the behavior of the increased compressive strength at the age of 28 days in cement mortar cubes prepared with microbial cells. There was a measurable increase in compressive strength of cement mortar cubes prepared with S. Pasteurii, supported by previous studies. Thus, it was concluded that the increase in compressive strengths is mainly due to consolidation of the pores inside the cement mortar cubes with microbiologically induced calcium carbonate precipitation. The performance of S. Pasteurii in urease production, calcite precipitation and improvement in compressive strength appears equally effective whether they are grown in media containing 10% LML or other nutrient media. Addition of S. Pasteurii has a positive effect on the compressive strength of cement mortar. Undoubtedly, S. Pasteurii not only provides a nucleation site for calcite precipitation but also creates an alkaline environment inducing further growth of calcite.

Conclusions: LML is a good source of nutrients that can support growth and urease activity of this bacterium. The present study results suggest that LML can serve as a better nutrient source for the growth of the bacteria and also for calcite precipitation. Use of LML in place of standard media serves as eco-friendly technology to prevent environmental pollution.

3.2.6 Waste water treatment plant (WWTP) sludge

WWTP Sludge is a by-product of the water cleanup process in waste water treatment plants. In Europe, before disposal, municipal sludge has to be treated to eliminate the bacteria, viruses and organic pollutants. The steps involved in a typical process are listed below:

- Preliminary treatment (screening, comminuting).
- Primary thickening (gravity, flotation, drainage, belt, centrifuges).

- Liquid sludge stabilization (anaerobic digestion, aerobic digestion, lime addition).
- Secondary thickening (gravity, flotation, drainage, belt, centrifuges).
- Conditioning (elutriation, chemical, thermal).
- Dewatering (plate press, belt press, centrifuge, drying bed).
- Final treatment (composting, drying, line addition, incineration, wet oxidation, pyrolysis, disinfection).
- Storage (liquid sludge, dry sludge, compost, ash).
- Transportation (road, pipeline, sea).
- Final destination (landfill, agriculture/horticulture, forest, reclaimed land, land building, other uses).

WWTP Sludge is generated during the primary (physical and/or chemical), the secondary (biological) and the tertiary (additional to secondary, often nutrient removal) treatment.

- Primary sludge produced by settleable solids removed from raw wastewater in primary settling: characterised by high putrescibility and good dewaterability when compared to biological sludge: Total Solids content in primary sludge is in the range 2-7% (Turovskiy and Mathai, 2006).
- Secondary sludge (biological sludge) produced by biological processes such as activated sludge or biofilm systems: contains microorganisms grown on biodegradable matter (either soluble or particulate), endogenous residue and inert solids not removed in the primary settling (where a primary settler is present) or entering with the raw wastewater (where no primary settler is present): TS content in secondary sludge is in the range 0.5-1.5% (Turovskiy and Mathai, 2006).
- Chemical sludge produced by precipitation of specific substances (i.e. phosphorus) or suspended solids.

The cost for treatment and disposal of sludge in European countries has been estimated to reach on average 500 € per tonne of dry mass, according to the type of treatment and disposal. (Sludge Reduction Technologies in Wastewater Treatment Plants, IWA Publishing: 2010). As a consequence, there are two aims with regard to sludge: The recovery of materials or energy from sludge, if sludge is considered a resource, and the reduction of the amount of sludge produced, if sludge is considered waste.

The sludge treatment process is described here: The wastewater enters large settling tanks where the oil and grease are removed after it floats to the top. Heavier material sinks to the bottom of the tank and is removed afterwards. Once the screening is performed and the grit is removed, the wastewater still contains light organic suspended solids. Some of these can be removed by gravity in a sedimentation tank. These tanks are typically twelve feet deep and hold the wastewater for two or three hours. What settles out is the sludge. The sludge is removed from the primary treatment tank with mechanical scrapers and pumps.

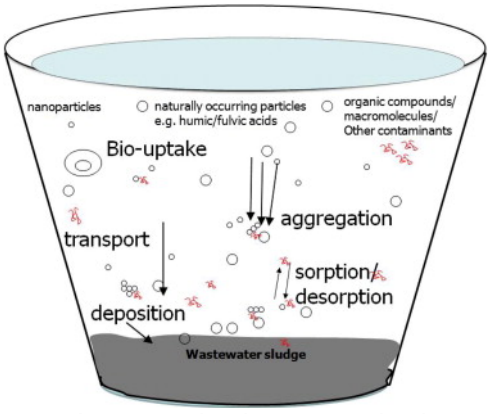

Figure 7 Hypothetical representation of mechanisms operating in a typical wastewater treatment plant process step (different mechanisms show further concentration into wastewater sludge)

Then, the sludge needs to be stabilized for what several methods can be employed. From them, lime addition to sewage sludge reaches a good level of stabilization by adding about 30 % of lime to the dry matter. Lime treatment of sludge therefore generates a product with a useful content in CaO. However, as the calcium content may be highly variable in lime treated sludge, it is generally necessary to analyse the sludge before use.

Secondary treatment is done by a method named activated sludge whereby a mixture of wastewater and biological sludge (microorganisms) is agitated and aerated. The biological solids are then allowed to settle out. The name "activated sludge" comes from the biological mass formed when oxygen (in the form of air) is continuously injected into the wastewater. In this process, microorganisms are thoroughly mixed with organics under conditions that stimulate their growth. As the microorganisms grow and are mixed by the agitation of the air, the individual microorganisms flocculate together to form a mass of microbes called activated sludge. About eight cubic feet of air are required for every cubic foot of wastewater.

The characteristics of sludge depend on the original pollution load of the treated water, and also on the technical characteristics of the treatment carried out. Water treatment concentrates the pollution present in water and therefore sludge contains a wide variety of matter, suspended or dissolved. Some compounds may be usefully reused (organic matter, nitrogen, phosphorus, potassium, calcium, etc.) whereas other compounds are pollutants (such as heavy metals, organic pollutants, and pathogens).

The sources of solids in a treatment plant vary according to the type of plant and its method operation. A typical chemical composition and properties of untreated and digested sludge is contained in the following Table:

Item/sludge	Untreated primary		Digested primary		Activated range
	Range	Typical	Range	Typical	
Total dry solids (TS), %	2.0-8.0	5.0	6.0-12.0	10.0	0.83-1.16
Volatile solids (% of TS)	60-80	65	30-60	40	59-88
Grease and fats (% of TS)					
Ether soluble	6-30	-	5-20	18	-

Ether extract	7-35	-	-	-	5-12
Protein (% of TS)	20-30	25	15-20	18	32-41
Nitrogen (N, % of TS)	1.5-4	2.5	1.6-6.0	3.0	2.4-5.0
Phosphorous (P_2O_5 of %TS)	0.8-2.8	1.6	1.5-4.0	2.5	2.8-11.0
Potash (K_2O_5 %TS)	0-1	0.4	0.0-3.0	1.0	0.5-0.7
Cellulose (% of TS)	8.0-15.0	10.0	8.0-15.0	10.0	-
Iron (not as sulfide)	2.0-4.0	2.5	3.0-8.0	4.0	-
Silica (SiO2, % TS)	15.0-20.0	-	10.0-20.0	-	-
Alkalinity (mg/l as CaCO3)	500-1500	600	2500-3500	3000	580-1100
Organic acids (mg/l as Hac)	200-2000	500	100-600	200	1100-1700
Energy content	10,000-12,500	11,000	4000-6000	5000	8000-10000
pH	5.0-8.0	6.0	6.5-7.5	7.0	6.5-8.0

Table 13 Typical chemical composition and properties of untreated/digested sludge (Source: Wastewater engineering: Treatment, disposal and reuse. 3rd Edition. Mc GrawHill)

The main groups of the organic solids in the sludge are protein, carbohydrates, fats and oils, which vary with their origin, system and efficiency of the wastewater treatment plant. The sludge can be a very good source of carbon, nitrogen, phosphorus and other nutrients for many microbial processes that can add to the value of sludge by producing valuable metabolic product. The control of pH levels, alkalinity, organic acid content, content of heavy metals, pesticides, hydrocarbons has to be controlled for further application.

Because of the physical–chemical processes that are involved in activated wastewater sludge treatment, sludge tends to accumulate heavy metals existing in the wastewater such as zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), lead (Pb), mercury (Hg) and chromium (Cr). Concentrations of heavy metals in sewage sludge may vary widely, depending on the sludge origins. Typical metal concentrations are indicated in the next Table:

Metal	Dry sludge (mg/kg)	
	Range	Median
Arsenic	1.1-230	10
Cadmium	1-3,410	10
Chromium	10-990,000	500
Cobalt	11.3-2490	30
Copper	84-17,000	800
Iron	1000-154000	17,000
Lead	13-26000	500
Manganese	32-9870	260
Mercury	0.6-56	6
Molybdenum	0.1-214	4
Nickel	2-5300	80
Selenium	1.7-17.2	5
Tin	2.6-329	14

Zinc	101-49,000	1700

Table 14 Typical metal content in waste water sludge: Source: Hsiau P, Lo S. Extractabilities of heavy metals in chemically-fixed sewage sludges. J Hazard Mater 1998: 58:73–82

- Microbial calcite precipitation with WWTP sludge. Literature review.

Waste-activated sludge from wastewater treatment plants that treat domestic wastewater contains a high protein ratio. This protein source has a high content of toxic heavy metals and other potential carcinogenic pollutants in the sludge and pretreatment methods need to be used to lower the metal content. We have found a reference in the literature to the use of WWTP sludge for the production of urease activity. WWTP sludge is largely comprised of whole cells, which resulted inaccessible to the growing microorganisms. Despite of that, initial attempts to lyse the cells were made by exposing them to 0.5 M NaOH for 20 minutes, followed by addition of H2SO4 to neutralise the cell extract to pH 8 (Schutte and Kula, 1990). However, this procedure produced an extract that did not sustain growth of S. Pasteurii (data not shown) so further investigation is required.

4.- SUPPLY-DEMAND ANALYSIS: AVAILABILITY OF THE INDUSTRIAL WASTE AS PROTEIN SOURCE

The following Table lists the sources of industrial by-product from which alternative sources of proteins can be obtained, together with the availability of the industrial by-product on each case.

Protein source	Source	State	Average production
Corn Steep Liquor (CSL)	By-product of corn wet milling industry	Viscous liquid	A plant processing 70,000 bushels (1,778 tones) of corn per day generates roughly 350,000 (1,324,890 liters) gallons of steepwater per day. (EPA. USA). 1 kilo of this sample of steep water, dry basis, is the concentrated extract from 13 to 16 kilos of corn.
Torula yeast (Candida utilis)	By-product of the paper industry, used for the purpose of reducing effluent sulfite liquor coming from the process	Light grayish-brown powder	Eight to 10 tons of sulfite waste liquor are produced per ton of paper pulp. On the waste liquor of each short ton of beech pulp produced, 200 to 240 lbs. (90 kgs) of yeast can be grown.
Brewery waste yeast	Brewing by-product recovered by natural sedimentation at the end of the second fermentation and maturation	Yellow powder	Excess yeast 2-4% of the total production volume
Sludge biomass from WWTP	By-product of waste water treatment plants	Black semi-solid material	In Europe, dry weight per capita production of sewage sludge resulting from primary, secondary and even tertiary treatment is in average 90 g per person per day.
Lactose Mother Liquor	Waste water generated by the lactose manufacturing milk plants	Yellow slurry	Every 10 liters of raw milk processed in the milk plant to produce lactose, generates 1 liter of lactose mother liquor

Table 15 Availability of the waste

Together with the resource availability is it is worth to have an estimation of the amount of protein that is needed for the bacterial growth. In that sense, Al-Thawadi (2008) suggested that the amount of protein content needed is 20 g. L^{-1} of yeast extract. If this amount is substituted by an alternative protein source this concentration would have to be increased. However, this will be determined in WP3.

Samples of the waste material can be requested to several industrial European Associations. These associations can serve as first contact point in order to get access to the industrial manufacturers. All of them are listed in the table below but more information can be found in Annex II of this report:

Waste	Company	Address	Country
Corn Steep Liquor (CSL)	Association of European Sweet Corn Processors AETMD	21, chemin de Pau 64121 MONTARDON Tel : +33 (0) 5 59 12 67 00 Fax : +33 (0) 5 59 12 67 10	France
Torula yeast (Candida utilis)	CEPI aisbl Confederation of European Paper	250 Avenue Louise, box 80 B-1050 Brussels Belgium Tel: +32 2 627 4911 Fax: +32 2 646 8137 Email: mail@cepi.org	Belgium
Surplus yeast from breweries	The Brewers of Europe	23-25 Rue Caroly B - 1050 Brussels Belgium Tel. : (32) 2 - 551 18 10 Fax : (32) 2 - 660 94 02 http://www.brewersofeurope.org info@brewersofeurope.org	Belgium
Lactose Mother Liquor (LML)	European Dairy Association (EDA)	14 rue Montoyer 1000 Brussels Belgium Tel: + 32 2 549 50 40 Tel: + 32 2 549 50 49 eda@euromilk.org www.euromilk.org	Belgium

Table 16 European Associations. Organisms to be consulted when applying for the required by-products

5.- POLITICAL AND LEGAL BARRIERS AT EU LEVEL

5.1 Corn Steep Liquor

CSL is a viscous liquid mixture consisting entirely of the water soluble components of corn steeped in water. All constituents are naturally occurring nutritive materials such as crude proteins, amino acids, vitamins, reducing sugars (e.g., dextrose), organic acids (e.g., lactic acid), minerals, and other elemental nutrients. CSL is mainly transported in the water, and its constituents are easily assimilated into normal cell metabolism. No ecological, mammalian or human toxicity would be expected from these natural nutritive materials. Exposure is not likely to result in adverse effects since all CSL components are natural to the corn except for the small amount of sulfur dioxide. Sources of aqueous sodium dioxide (e.g., sulfur dioxide, sodium bisulfite) are unlikely to cause any adverse effects. Exposure during these uses would be mitigated by automation and wearing normal laboratory personal protective equipment.

The European Union allows the use of stillage and stillage extracts as fertilizers and soil conditioners in organic crop production, however, corn steep liquor is not mentioned specifically (European Union, 2008). Maize bran and gluten from wet corn milling are permitted as feed materials used in livestock production (European Union, 2008). European manufacturers refer to corn wet milling as maize processing. The processes are the same, including the use of sulfur dioxide. The Codex Alimentarius allows the use of stillage and stillage extracts as fertilizers and soil conditioners in organic crop production, however, corn steep liquor is not mentioned specifically (Codex Alimentarius, 137 2008). Corn steep liquor is included on the chemical inventory of the Domestic Substances List by the Canadian government. CSL is not a dangerous substance in accordance to the Council regulation (CE) No. 1272/2008 and the Directive 67/548/CEE and 1999/45/CE. Also, it has not been classified as hazardous in the European Waste Catalogue.

5.2 Torula yeast (Candida Utilis)

Traditionally, the Torula yeast has been used as a supplement in cattle diet. The author has not identified any regulation related to other uses. Since the Community law does not include specific provisions on the use of substances other than vitamins or minerals in food supplements, the free movement of this product is governed by Articles 28 to 30 of the EC Treaty and can thus be subjected to national restrictions or bans within the limits laid down by Article 30.

Their use as food supplements, is covered by legislative texts of general application in the area of food safety legislation, some of which were adopted or entered into force subsequent to the adoption of Directive 2002/46/EC:

- Regulation (EC) No 178/2002: Regulation (EC) No 178/2002 lays down the general legal framework and requirements of food law and the procedures applicable in the area of food safety.
- Regulation (EC) No 258/977 on Novel Foods. The objective of this Regulation is to make subject to an authorization procedure, and thus to a safety assessment, all foods and food ingredients covered by its scope, i.e. those covered by the definition of “novel food” or “novel ingredient”.

- Regulation (EC) No 1924/200610 on nutrition and health claims This Regulation lays down the conditions for the use of nutrition and health claims on food packaging.
- Regulation (EC) No 1925/200611 on the addition of vitamins and minerals and of certain other substances to foods.
- Directive 82/471/CEE relative to certain products used in animal feed.

Torula yeast is not a dangerous substance in accordance with the Council regulation (CE) No. 1272/2008 and the Directive 67/548/CEE. Also, it has not been classified as hazardous in the European Waste Catalogue.

5.3 Brewery waste yeast (Surplus yeast)

The surplus yeast has been used as a supplement for animal feed and the author has not identified any regulation related to other uses. Since the Community law does not include specific provisions on the use of substances other than vitamins or minerals in food supplements, the free movement of this product is governed by Articles 28 to 30 of the EC Treaty. As food supplements, is covered by legislative texts of general application in the area of food safety legislation, some of which were adopted or entered into force subsequent to the adoption of Directive 2002/46/EC:

Brewers pay for the water which is used for the brewing process. They also pay for treating the water after it has been used. Wastewater is treated in compliance with the Water Framework Directive (WFD) (2000/60/EC) and the Urban Water Directive (91/271/EEC). The WFD aims for all water bodies to reach at least good status by 2015 and follows a “polluter pays” policy, while the Urban Water Directive provides more specific guidelines for industries.

Surplus yeast is not a dangerous substance in accordance with the Council regulation (CE) No. 1272/2008 and the Directive 67/548/CEE. Also, it has not been classified as hazardous in the European Waste Catalogue.

5.4 Sludge biomass from WWTP

A number of directives on waste treatment have been approved by the European Union, as follows:

- As early as 1975, the waste framework directive required Member States to manage waste by encouraging prevention and environmentally friendly disposal.
- The Sewage Sludge Directive 86/278/EEC seeks to encourage the use of sewage sludge in agriculture. At the same time it regulates its use in such a way that any potential harmful effect on soil, vegetation, animals and human beings is prevented. According to the above principle, the use of untreated sludge in agriculture is prohibited, unless it is injected or incorporated in the soil. Moreover, by the term treated sludge is defined the sewage sludge which ‘‘has undergone biological, chemical or heat term, long-term storage or any other appropriate process so as significantly to reduce its ferment ability and the health hazards resulting from its use’’. The EU is currently assessing whether this Directive should be reviewed. Also, the future biological treatment on biodegradable waste Directive will establish the revised limits for heavy metals for sludge reuse.
- The hazardous waste directive in 1991 set the rules for handling this type of waste.

- The Urban Waste Water Directive 91/271/EEC was amended by 98/15/EC sets more stringent quality standards for waste waters. The main article of the Urban Waste Water Treatment Directive dealing with sludge is Article 14, where it is declared that ‘‘sludge arising from waste water treatment shall be re-used whenever appropriate’’. In addition, Article 14 also obliges Member States to ‘‘ensure that by 31 December 1998 the disposal of sludge to surface waters by dumping from ships, by discharge from pipelines or by other means is phased out’’.
- The European Union’s has established the target to reduce final waste disposal by 50% at 2050. To do this, it has drawn up a strategy setting the following priorities: (a) prevention of waste: (b) waste recovery through, reuse, recycling and energy recovery: (c) improved treatment conditions: (d) regulation of transport.
- Legislation concerning emission limits for individual plants/processes (and also fuel quality standards limiting the content of certain compounds in fuel).
- Emissions limits or ceilings at a national level
    - Legislation for local air quality concentration limits (often concerning local plant or processes).
    - National or European air quality concentration limits, mandating threshold levels to be met at ambient background areas (objectives or standards).
    - National economic instruments (energy or pollution taxes and charges).

5.5 Lactose Mother Liquor

Lactose mother liquor is very high strength waste water generated by the lactose manufacturing milk plants. Is has not been classified as a hazardous substance according to the European waste Catalogue. The author has not identified any other specific reference relative to the product in the EU legislative sources consulted.

6.- EVALUATION OF CALCITE PRECIPITATION

The cementation process covers the use of recycled cement as well as possible alternative fillers or alternative feed for the microorganism. In the best case, the complete process manages the formation of cement made of waste. According to the cultivation of the microorganism *S. Pasteurii*, the first cementation trials were performed with *S. Pasteurii* cells. Several conditions were tested:

- Temperature
- Grain type
- Grain size
- Cell density (OD)
- Culture
- Geometry of the trial

There exists a strong dependence for the calcite precipitation on these parameters. The protocol for evaluation of the calcite precipitation is described in Annex I and focuses at first on the mixing time, the cell concentration and $CaCl_2$ concentration. The value of interest is the cementation rate. According to the protocols, which CNR and IFAM will follow, the calcite precipitation will be estimated and correlated to the grain consolidation.

In general, the aspects that should be considered in preparation of the ECO-CEMENT are listed the next table:

Aspect	Description	Relevant impact	
OD	See Stressors in Table 3	Physical properties (e.g. strength, water uptake, resistance towards temperature stress)	Preparation in Lab scale and scale up options, CO_2 impact, urease activity, etc.
Filler	Type (e.g. Sand)		
	Grain size distribution (includes the shape of the filler as well as the grading curve)		
	Hardness of the Filler	Cracks run different depending on this difference (filler harder than matrix or the other way around)	
	Hardness of the matrix (matrix is represented by the calcite, the bacteria cell and the remaining medium/ cementation solution)		
Cementation solution	Concentration	Physical properties	
	Mixture (blend of Ca^{2+} source, Urea, CASO, yeast,…waste)		
Geometry	Shape of the negative form (e.g. cube → carton moulds, petri dishes, sweet silicone moulds) For cylindrical shape then take into account the aspect ratio 1:4 (diameter: height)	Edge effects!	Tests
	Diffusion of oxygen	Determines the edge length	

	Duration of hardening	Determines the application	
Tests	Mechanical Tests, Water uptake		Application

Table 17 Critical parameters affecting the precipitation of calcite.

7.- CONCLUSIONS

This Deliverable has explained that a reduction in the medium costs without loss of urease activity is possible by the substitution of laboratory grade yeast extract with different industrial by-products: Corn Steep Liquor (CSL); Torula Yeast; Brewery waste yeast (BWY); Sludge Biomass from WWTP, and Lactose Mother Liquor (LML). Each of them has advantages and disadvantages when compared to the others.

To assist in the selection process, the following table ranks them according to the 5 requirements needed for the culture media; high protein content, good availability, low or zero-cost, no additional processing, and high levels of urease activity being produced. (Information extracted from report).

The lowest number [1] indicates the best option and the highest number [4] indicates the worst option. A repeated number means that both residues hold the same position in the rank. Therefore, *the lower score the better the option.*

Industrial By-product	Protein %	Availability	Cost	Additional processing	Urease Activity (literature review)	∑ Total
CSL	3	4	2	1	1	11
Torula Yeast	2	3	3	2	2	12
BWY	1	1	2	2	2	8
Sludge wwtp	3	2	1	3	3	12
LML	4	1	1	1	1	8

Considering our criteria, ***Lactose Mother Liquor, Brewery waste yeast and, to a lesser extent, Corn Steep Liquor*** *are the wastes that suit better the project requirements. Torula yeast and Sludge biomass are discarded based on the above assumptions.* However, other facts have to be considered:

Why Torula yeast and Sludge biomass from WWTP have been discarded?

- Torula yeast (Candida utilis). The author has found just one reference in the literature related to microbial calcite precipitation by the use of Torula yeast. The results were not completely satisfactory. Further research is needed in order to assess the urease activity produced by the use of Torula yeast. However, the project has time limit constraints. Given that, Torula yeast is not recommended as a first option to form an alternative medium for the bacterial growth.
- Sludge biomass from WWTP. This by-product has a high content in toxic heavy metals and other potential carcinogenic pollutants so pretreatment methods are needed to lower the metal content in the sludge. These pretreatment methods add an extra cost that should be avoided. For this reason, we have discarded Sludge biomass as a cost-effective alternative.

Which is the best option between; CSL, BWY and LML?

- Corn Steep Liquor (CSL). In Section 3.2.2 it has been demonstrated that the corn steep liquor is a suitable nutrient source for the growing bacteria as it produces good levels of urease activity. However, it is not widely available in Europe, so other industrial by-products had to be considered with better availability and similar enzymatic results.
- Brewery waste yeast (BWY). Several attempts have been done aiming to reuse the surplus yeast in biotechnological processes as a source of nutrients. However, brewery waste yeast is largely comprised of whole cells that resulted inaccessible to the growing microorganisms but this could be improved in further investigation. There are other industrial by-products that suit better the project requirements. Brewery waste yeast is not recommended as a first option to form an alternative medium for the bacterial growth.
- Lactose Mother Liquor (LML). This report suggests that LML can serve as the better nutrient source for the growth of the bacteria and also for calcite precipitation. LML is a good source of nutrients that can support growth and urease activity of *Sporosarcina Pasteurii*. Using LML instead of standard media does not only reduce the cost but also serves as eco-friendly technology to prevent environmental pollution. It is very important to note that previous experiences have demonstrated that the final level of urease activity was sufficient for cementation. The availability of this waste is guaranteed by the production regularity and the great amount of manufacturing industries all around Europe.

In conclusion, despite lower protein content this Deliverable suggests that ***LML is better nutrient source for the growth of the bacteria and also for calcite precipitation*** .The availability of this waste is guaranteed by the abundance of manufacturing industries all around Europe. Samples of this material will be provided in order to conduct the laboratory tests in WP3 for our specific project.

Urea can also be substituted by alternative and cheaper sources. In section 3.1 we have indentified viable and available sources of less expensive urea that could be employed in ECO-CEMENT. Traditional sources of urea include; excretory products (faeces and urine) and other sources (sewage). Nonetheless, the typical values are not representative. This suggests that an ***accurate assessment is needed in a case by case basis.*** For that, the following factors should be taken into account: content of urea; cost; availability of the waste.

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9.- ANNEX I: ECO-CEMENT PRELIMINARY PROTOCOLS

A. $CaCo_3$ precipitation
B. Grains consolidation

A. $CaCo_3$ precipitation

Day 1 (18:00)

1. Growth of urease active bacteria in nutrient medium

CASO broth 200 ml + 20 g/L urea + 0.2 ml of bacteria inoculum (2x 10^8 bacterial cells - DO) incubate at 30°C, rotor shaking 150rpm, overnight (till the late exponential stage)

Parameters to be controlled:
- $NH4^+$ (Nessler – Conductivity)
- microbial biomass (CFU, OD)
- pH

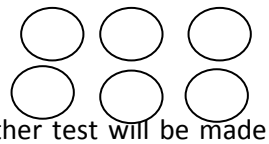
Day 2 (08:00)

2. Initialization and monitoring of $CaCO_3$ precipitation

Set of small flasks containing 0.11 ml (or 1.1 ml) of a $CaCl_2$ 2.52 M stock solution (two triplicate set per each time of reaction + one double set with distilled water) and 10.89 ml (or 9.9 ml) of the CASO broth with overnight bacterial growth into each flask, to reach a 25.2 mM (or 252 mM) $CaCl_2$final concentration, then, rotor shaking 150 rpm at 30°C. Use one set at different time of reaction (after 3, 6, 9 and 24h).
- measure parameters in each set

For each time of reaction the content of each set of flask (11 ml) will be handled as following:
- 1 ml of each flask for OD measurement
- the rest 10 ml will be centrifugated, filtered, dried and weighted in order to evaluate the amount of CaCO3 precipitated

Example: the 2 triplicate set of flasks (centrifugated and then filtered), used at one time monitoring. The supernatant



As such
After HCl (0.1M) treatment

Further test will be made determining the amount of the new carbonate by XRD (**ICVBC**) or EDTA titration.

B - Grains consolidation by microbial CaCo3 precipitation

Day 1 (18:00)

1. Growth of urease active bacteria in the nutrient medium

CASO broth 200 ml + 20 g/L urea + 0.2 ml of bacteria inoculum (2x 10^8 bacterial cells - DO) incubate at 30°C, rotor shaking 150rpm, overnight (till the late exponential stage)

Parameters to be controlled:
- $NH4^+$ (Nessler – Conductivity)
- microbial biomass (CFU, OD)
- pH

Day 2 (09:00)

2. Microbial/sand-stonepoultice formation

Add slowly into the CASO broth with overnight bacterial growth the sand (or stone) grains (using 2 classes of grain sizes, for example

i) 0.25-0.4 µm

ii) 0.4-0.63 µm),

Continuously mixing, until the formation of a creamy poultice (to be experimented the liquid/solid ratio, because it is depending on type of solid grains). Add the 2 ml (or 20 ml) $CaCl_2$ solution (to reach the final concentration of 25.2 mM or 250 mM, respectively), drop by drop, continuously mixing, for few minutes (5-10', for example), and then, pour the creamy poultice into a set of moulds and fill them (Petri dishes or sweets silicone moulds for example) for different thickness of the poultice 0.5 – 1.0 – 2.0 cm.

Day X1,X2,X3 (09:00)

3. Cementation

Let the poultice to endure into the moulds for X1 – X2 – X3 days at room temperature and then pull out the "bricks" (or other conditions – such as oven, climatic chamber with controlled T° and RH).

Parameters to be controlled for each variation made (type of stone -grain size –Cacl2 conc. Time of mix – thickness of the brick – time of cementation

- measure the hardness (superficial and in depth by sonication, peeling test and other tests)
- evaluate the difference in water absorption before and after sonication

10.-ANNEX II: ALTERNATIVE WASTE SUPPLIERS

Waste	Company	Address	Country
Corn Steep Liquor (CSL)	Association of European Sweet Corn Processors AETMD	21, chemin de Pau 64121 MONTARDON Tel : +33 (0) 5 59 12 67 00 Fax : +33 (0) 5 59 12 67 10	France
	Cargill, Incorporated	Haubourdin, Pas-de Calais	France
		Krefeld, Nordrhein-Westfalen	Germany
		Castelmassa, Veneto	Italy
		Martorell, Barcelona	Spain
		Orhangasi, Bursa	Turkey
	Roquette America, Inc.	Lestrem, Pas-de-Calais	France
		Beinheim, Bas-Rhin	France
		Cassano Spinola, Alessandria	Italy
		Calafat, Dolj	Romania
		Benifayo, Valencia	Spain
	Tate & Lyle Americas	Razgrad	Bulgaria
		Koog aan de Zaan	Netherlands
		Bolera	Slovakia
		Adana	Turkey

Waste	Company	Country
Top European Paper Industries: Sulfite waste slurry -> Torula yeast (Candida utilis)	Svenska Cellulosa (SCA)	Sweden
	Stora Enso	Finland
	UPM-Kymmene	Finland
	Smurfit Kappa	Ireland
	Metsaliitto	Finland
	Mondi group	UK
	Sequana Capital	France
	Norske Skog	Norway
	DS Smith	UK
	Burgo	Italy
	Holmen	Sweden
	Ahlstrom	Finland
	Sonae Industria	Portugal
	Sodra	Sweden
	The Lecta Group	France
	Pfeiderer	Germany
	Mayr-Melnhof Karton	Austria

	Myllykoski	Finland
	Portucel	Portugal
	Moelven	Norway
	Billerud	Sweden
	Kinnevik (Korsnas)	Sweden
	Sveaskog	Sweden
	ENCE	Spain
	Groupe Gascogne	France
	Heinzel Holding	Austria
	Exacompta Clairefontaine	France
	Setra Group	Sweden
	Corticeira Amorim	Portugal
	Reno de Medici	Italy

Waste	Company	Country
Paper Industries Associations	CEPI aisbl Confederation of European Paper Industries	250 Avenue Louise, box 80 B-1050 Brussels Belgium Tel: +32 2 627 4911 Fax: +32 2 646 8137 Email: mail@cepi.org
	AUSTROPAPIER Vereinigung der Österreichischen Papierindustrie	Gumpendorfer Strasse 6 A-1061 Vienna T: +43 1 588 86 0 F: +43 1 588 86 222 austropapier@austropapier.at www.austropapier.at
	COBELPA Association des Fabricants de Pâtes, Papier et Cartons de Belgique	Avenue Louise 306, Box 11 B-1050 Brussels T: +32 2 646 64 50 F: +32 2 646 82 97 general@cobelpa.be www.cobelpa.be
	ACPP Asociace ceskeho papirenskeho prumyslu - Association of the Czech Pulp and Paper Industry	Litomericka 272 CZ-411 08 Steti T: +420 416 803 934 F: +420 416 803 935 acpp@acpp.cz www.acpp.cz
	FFIF Finnish Forest Industries Federation Snellmaninkatu	Snellmaninkatu 13 FIN-00170 Helsinki PO Box 336 FIN-00171 Helsinki T: +358 9 132 66 00

	F: +358 9 132 44 45 name.surname@forestindustries.fi www.forestindustries.fi
COPACEL Confédération Française de l'Industrie de Papiers, Cartons et Celluloses	23-25 rue d'Aumale F-75009 Paris T: +33 1 53 89 24 00 F: +33 1 53 89 24 01 contacts@copacel.fr www.copacel.fr
VDP Verband Deutscher Papierfabriken	Adenauerallee 55 D-53113 Bonn T: +49 228 267 05 0 F: +49 228 267 05 62 vdp.bonn@vdp-online.de www.vdp-online.de
FEDPRINT Federation of the Hungarian Printers and Paper Makers	Bartok Bela ut 41 H-1114 Budapest T: +36 1 350 77 28 F: +36 1 350 77 27 office@fedprint.hu www.fedprint.hu
ASSOCARTA Associazione Italiana fra gli Industriali della Carta, Cartoni e Paste per Carta	Bastioni di Porta Volta 7 I-20121 Milano T: +39 02 290 03 018 F: +39 02 290 03 396 Viale Pasteur 8-10 I-00144 Roma T: +39 06 591 91 31 F: +39 06 591 08 76 assocarta@assocarta.it www.assocarta.it
Royal VNP Vereniging van Nederlandse Papier- en kartonfabrieken	Kruisweg 761 NL-2132 NE Hoofddorp PO Box 731 NL-2130 AS Hoofddorp T: +31 20 654 30 55 F: +31 20 654 30 64 info@vnp-online.nl www.vnp-online.nl
Norsk Industri	Middelthunsgate 27 N-0306 Oslo PO Box 7072 Majorstuen N-0306 Oslo T: +47 23 08 88 00 F: +47 23 08 88 98 post@norskindustri.no www.norskindustri.no

	SPP Association of Polish Papermakers	Plac. l. Komuny Paryskiej 5A PO Box 200 PL-90-950 Lódz´ T: +48 42 630 01 17 F: +48 42 632 43 65 info@spp.pl www.spp.pl ul. Al. Jerozolimskie 44: room: 1126 PL-00-024 Warszawa T: +48 22 433 61 20 F: +48 22 433 61 20 biuro@spp.pl
	CELPA Associação da Indústria Papeleira	Rua Marquês de Sá da Bandeira 74 - 2° P-1069 - 076 Lisboa T: +351 2 1 761 15 10 F: +351 2 1 761 15 11 celpa@celpa.pt www.celpa.pt
	ROMPAP The Patronizing Organization for Romanian Pulp and Paper Industry	Piata Walter Maracineanu 1-3 Intr. 2, Et. 2, Cam. 177-178 RO-Sector 1 - Bucarest T: +40 21 315 01 62 F: +40 21 315 01 75 rompap.romania@gmail.com
	ZCPP SR Union of Pulp and Paper Industry of the Slovak Republic	Ticha´ 30 SK-974 01 Banska´ Bystrica T: +421 48 412 37 76 F: +421 48 412 37 76 info@paper.sk www.paper.sk
	Chamber of Commerce and Industry of Slovenia Paper and Paper Converting Association	Dimiceva 13, SI-1504 Ljubljana T: +386 1 58 98 000 F: +386 1 58 98 100 info@gzs.si www.gzs.si
	ASPAPEL Asociación Española de Fabricantes de Pasta, Papel y Cartón	Avenida de Baviera 15 E-28028 Madrid T: +34 91 576 30 03 F: +34 91 577 47 10 aspapel@aspapel.es www.aspapel.es
	SFIF Swedish Forest Industries Federation	Storgatan 19 PO Box 55525 SE- 102 04 Stockholm

		T: +46 8 762 72 60 F: +46 8 611 71 22 info@forestindustries.se www.forestindustries.se
	CEPI Confederation of Paper Industries	1 Rivenhall Road Swindon UK - Wiltshire SN5 78D T: +44 1 793 88 96 00 F: +44 1 793 87 87 00 cpi@paper.org.uk www.paper.org.uk

Waste	Company	Country/Address
Surplus yeast from breweries	The Brewers of Europe	The Brewers of Europe 23-25 Rue Caroly B - 1050 Brussels Belgium Tel. : (32) 2 - 551 18 10 Fax : (32) 2 - 660 94 02 http://www.brewersofeurope.org email : info@brewersofeurope.org
	APCV - Associação Portuguesa dos Produtores de Cerveja	PORTUGAL Edificio Empresarial EE3 Pólo Tecnológico de Lisboa, Lote 3, 1600-546 Lisboa Tel. (351) 21 710 1777 Fax. (351) 21 710 1795 Président : Antonio Pires de Lima Secrétaire Général : Francisco Girio Email: apcv@lispolis.pt www.apcv.pt
	Asociatia Berarii Romaniei	ROMANIA Modern Business Center Bdul Carol I, nr 34-36, etaj 2, sector 2, Bucuresti 020922 Phone: +40 21 317 29 77 Fax: +40 317 29 85 President: Mr. Hezy Ovadia General Manager: Mr. Constantin Bratu Email: info@berariiromaniei.ro www.berariiromaniei.ro
	Association of Hungarian Brewers	HUNGARY

		Margitsziget Grand Hotel 4th floor 1138 - BUDAPEST Hungary Phone: +36 1 486 0536/537 Fax: +36 1 266 3661 President : Ms Klára Csík Email: mssz@sorszovetseg.hu www.sorszovetseg.hu
	Associazione degli Industriali della Birra e del Malto	ITALY Viale di Val Fiorita, 90 I - 00144 ROMA Tel. : (39) 06 54 393 201 Fax : (39) 06 591 29 10 Président : Dr. Alberto Frausin Secretary General : Filippo Terzaghi Email: segreteria@assobirra.it www.assobirra.it
	Beer and Malt Producers´ Association of Turkey	TURKEY Selanik Caddesi No: 44/1 TR - KIZILAY ANKARA Tel : (90) 312 419 35 18 Fax : (90) 312 419 38 79 President : Mehmet Can Karakaş Secretary General : Nejat Eren Email: info@bmud.org.tr www.biramalt.com
	Belgian Brewers	BELGIUM Maison des Brasseurs Grand´Place 10 B - 1000 BRUXELLES Tel. : (32) 2 511 49 87 Fax : (32) 2 511 32 59 Président : Theo Vervloet Directeur Général : Sven Gatz Email: info@belgianbrewers.be www.belgianbrewers.be
	Brasseurs de France	FRANCE Boulevard Malesherbes, 25 F - 75008 PARIS Tel. : (33) 1 42 66 29 27 Fax : (33) 1 42 66 52 79 Président - Délégué Général : Gérard Laloi Secrétaire Général : Louis Delalande Email: contact@brasseurs-de-france.com

		www.brasseurs-de-france.com
	British Beer and Pub Association	UNITED KINGDOM Ground Floor Brewers' Hall Aldermanbury Square London EC2V 7HR Tel. (44) 207 627 9191 Fax (44) 207 627 9123 Chairman : Mark Hunter Secretary General : Brigid Simmonds OBE Email: enquiries@beerandpub.com www.beerandpub.com
	Bryggeriforeningen	DENMARK Faxehus Gamle Carlsberg Vej 16 DK - 1900 København V Tel. : (45) 72 16 24 24 Fax : (45) 72 16 24 44 President : Carsten Händel Director : Niels Hald Email: info@bryggeriforeningen.dk www.bryggeriforeningen.dk
	Cerveceros de España	SPAIN c/. Almagro 24, 2° Izda. E - 28010 MADRID Tel. : (34) 91 308 67 70 Fax : (34) 91 308 66 61 Président : Juan Gervás Sanz Directeur Général : Jacobo Olalla Marañon Email: info@cerveceros.org www.cerveceros.org
	Croatian Chamber of Commerce Association of beer, malt and hop producers	CROATIA Rooseveltov trg 2 HR - 10000 Zagreb Tel. : (385) 1 456 1 555, 4561 643 Fax : (385) 1 456 15 45 President : Pero Ivanković Secretary: Sandra Tankosić Email : stankosic@hgk.hr www.hgk.hr
	Cyprus Brewers Association	Cyprus P.O.Box 21455 1509 Nicosia Cyprus Tel : (+357) 22 88 98 00

	Fax : (+357) 22 66 56 85 President : Kostas Koutsos Secretary : Nikos Georgiades Email: n.georgiades@keopgroup.com
Czech Beer and Malt Association	CZECH REPUBLIC Lipova 15 CZ - 120 44 Praha, 2 Tel : (420) 224 910 641 Fax : (420) 224 914 542 President : Frantisek Šámal Secretary General : Jan Vesely Email: jan.vesely@cspas.cz Web: www.cspas.cz
Deutscher Brauer-Bund e.V.	GERMANY Neustädtische Kirchstraße 7A D - 10117 BERLIN Tel: (49) 30 209167-0 Fax: (49) 30 209167-99 Präsident : Dr. Hans-Georg Eils Hauptgeschäftsführer : Peter Hahn Email: info@brauer-bund.de www.brauer-bund.de
Fédération des Brasseurs Luxembourgeois	LUXEMBURG Rue Alcide de Gaspéri, 7 B.P. 1304 L – 1013 Luxembourg – Kirchberg Tel. : (352) 43 53 66 1 Fax : (352) 43 23 28
Greek Brewers´ Association	GREECE 107 Kifissou av GR - 122 41 EGALEO Tel. (30) 210 538 42 87 Fax (30) 210 538 44 37 President : Jac van Herpen Secretary : Athanasios Syrianos Email: a.syrianos@eza-beers.gr
Lithuanian Breweres´ Guild	LITUANIA A.Tumėno 4, LT - Vilnius 01009 Lithuania Tel : (370) 52 498 495 Secretary General : Saulius Galadauskas Email: saulius.galadauskas@aludariai.lt
Nederlandse Brouwers	HOLLAND

	Dagelijkse Groenmarkt 3-5 NL - 2513 AL Den Haag PB 179 NL-2501CD Den Haag Tel. (31) 70 31 80 710 Fax (31) 70 31 06 173 President : Hans Wiegel Director : Cees-Jan Adema Email: info@cbk.nl www.nederlandsebrouwers.nl
Norwegian Brewers	NORWAY Sorkedalsveien, 6 P.O.Box 7087 Majorstuen N-0306 OSLO Tel. (47) 23 08 86 96 Fax (47) 22 60 30 04 President : Harald Bredrup Director : Petter Nome Email: info@bryggeriforeningen.no www.bryggeriforeningen.no
Panimoliitto	FINLAND Pasilankatu 2- P.O.Box 115 FIN - 00241 HELSINKI Tel. : (358) 91 48 87 1 Fax : (358) 91 48 87 201 President : Pekka Tiainen Managing Director: Elina Ussa Email: info@panimoliitto.fi www.panimoliitto.fi
Slovak Beer and Malt Association	SLOVAKIA Piva a sladu Zahradnicka 21 811 07 Bratislava President: Julia Hurna Email: pivoslad@euroweb.sk
Sveriges Bryggerier AB	SWEDEN Storgatan 19 S - 114 46 STOCKHOLM Tel. : (46) 8 762 6550 Fax : (46) 8 522 535 90 President : Mr. Paul Bergqvist Secretary General : Cecilia Giertta Email: info@sverigesbryggerier.se www.sverigesbryggerier.se
Swiss Breweries´ Federation	SWITZERLAND Engimattstrasse 11 P.O. Box 2124

		CH - 8027 ZURICH Tel. : (41) 44 221 26 28 Fax : (41) 44 211 62 06 President : Dr M. Zemp Secretary General: Marcel Kreber Email: info@bier.ch www.bier.ch
	The Irish Brewers´ Association	IRELAND 84/86 Lower Baggot Street IRL - DUBLIN 2 Tel. : (353) 1 6051558 Fax : (353) 1 6381558 Director : Thomas Burke Email: thomas.burke@ibec.ie www.abfi.ie
	The Malta Chamber of Commerce, Enterprise and Industry	MALTA Exchange Buildings, Republic Street Valleta VLT 1117 MALTA Tel.: (356) 21233873 Fax.: (356) 21245223 President : Martin Galea Director General : Ing. Ray Muscat Email: info@maltachamber.org.mt www.maltachamber.org.mt
	The Union of Brewing Industry Employers in Poland - Polish Brewers	POLAND Biuro Zarzadu Zwiazku Al. Jana Pawla II 12 lok. 339 00-124 Warszawa Tel: (48) 22 850 91 15 Fax: (48) 22 850 91 14 President: Robert Priday Director: Danuta Gut Email: biuro@browary-polskie.pl www.browary-polskie.pl
	Union of Brewers in Bulgaria (UBB)	BULGARY Union of Brewers in Bulgaria (UBB) 16 “Bacho Kiro” Street 1000 Sofia Tel/fax +359 2 989 40 24 President: Alexander Grancharov Secretary general: Ivana Radomirova Email: ubb@i-n.net www.pivovari.com
	Verband der Brauereien Österreichs	AUSTRIA

	Zaunergasse 1-3 A - 1030 WIEN Tel. (43) 1 713 15 05 Fax (43) 1 713 39 46 Präsident : Siegfried Menz Geschäftsführer : Jutta Kaufmann-Kerschbaum Email: getraenke@dielebensmittel.at www.bierserver.at

Waste	Company	Country
Lactose Mother Liquor (LML)	European Dairy Association (EDA)	14 rue Montoyer 1000 Brussels Belgium 00 32 2 549 50 40 00 32 2 549 50 49 eda@euromilk.org www.euromilk.org
	VÖM - Austrian Dairy Association	Friedrich-Wilhelm-Raiffeisen-Platz 1/11 A - 1020 Wien +43 1 211 36 25 75 +43 1 211 36 20 81 http://www.voem.or.at voem@netway.at
	BCZ-CBL - Belgian Dairy Industry Association	Hungaria Building 31/02.02 Vaartkom B - 3000 Leuven +32 16 30 07 70 +32 16 30 07 79 http://www.bcz-cbl.be office@bcz-cbl.be
	Bulgarian Association of Dairy Processors	Lagera complex Bl. 44 entr. A BG - 1612 Sofia +359 2 952 3265 +359 2 953 2723 http://www.milkbg.org/ bam@mb.bia-bg.com
	CMSM - Czech & Moravian Dairy Association	V Olsinách 75 CZ - 100 98 Praha 10 +420 2 7482 20 02 +420 2 7482 17 59 http://www.cmsm.cz/ cmsm@volny.cz

Danish Dairy Board (Mejeriforeningen)	22 Frederiks Allé DK - 8000 Arhus C +45 87 31 20 00 +45 87 31 20 01 http://www.mejeri.dk info@mejeri.dk
Estonian Dairy Association	J. Vilmsi 53 EST - 10147 Tallinn http://www.piimaliit.ee/ piimaliit@piimaliit.ee
Finnish Dairies' Association	Pasilankatu 2 P.O. Box 115 FI - 00241 Helsinki +358 9 148 871 +358 9 148 872 01 http://www.etl.fi info@etl.fi
ATLA - French Milk Processors' Association	Maison du Lait 42 rue de Châteaudun FR - 75314 Paris Cédex 09 +33 1 49 70 72 72 +33 1 42 80 63 62 http://www.maison-du-lait.com/QuiFait/atla/atla.html com@atla.asso.fr
DRV Deutscher Raiffeisenverband - Berlin	Postfach 080549 D - 10005 Berlin +49 30 856214-3 http://www.raiffeisen.de/ info@drv.raffeisen.de
SEVGAP - Hellenic Association of Milk & Dairy Products Industry	21, Agias Sofias Str. GR - 15451 Neo Psychiko - Athens +30 21 0671 11 77 +30 21 0671 10 80 sevgap@sevt.gr
IDIA - Irish Dairy Industries Association	Confederation House 84-86 Lower Baggot Street IRL- Dublin 3 +353 1 660 10 11 +353 1 661 28 70 http://www.ibec.ie info@ibec.ie
ASSOLATTE (Associazione Italiana Lattiero Casearia)	Via Adige 20 I - 20135 Milano +39 02 72 02 18 17 +39 02 72 02 18 38 http://www.assolatte.it assolatte@assolatte.it
Latvian Dairy Committee	Bauskas iela Nr 180 LV - 1004 Riga

	+371 762 05 46 +371 917 99 89
A.L.L. Luxemburg Dairy Association	7, Rue Alcide de Gasperi, BP 1304 L - 1013 Luxemburg info@luxlait.lu
NZO - Dutch Dairy Association	NZO - Dutch Dairy Association (Nederlandse Zuivel Organisatie) P.O. Box 165 NL - 2700 AD Zoetermeer +31 79 343 03 00 +31 79 343 03 20 http://www.nzo.nl info@nzo.nl
ZPPM - Association of Polish Dairy Processors	ul. Zlota 59, budynek Lumen, pietro 6 PL - 00-120 Warszawa +48 222 660 271 +48 222 660 327 http://www.zppm.pl sekretariat@zppm.pl
FENALAC (Feredacào Nacional das Uniones de Leite e Lacticinios)	Rua da Restauraçào 312 - 1e P - 4050-501 Porto +351 22 609 08 00 +351 22 600 09 91 www.fenalac.pt
Nacionalno udruženje preradjivaca mleka (Serbian Dairies Association - SEDA	Augusta Cesarca 18, V floor, office 510, 21000 Novi Sad, Republic of Serbia. 21000 Novi Sad www.serbiandairies.org
Slovak Dairy Union (Slovensky mliekarensky zvaz)	Záhradnícka 21 SK - 811 07 Bratislava +421 2 554 10945 +421 2 554 10945 http://www.smz.sk nouzovska@smz.sk
Slovenian Dairy Association	Dimiceva 9 SLO - 1000 Ljubljana +386 01 566 15 50 +386 01 566 15 49 http://www.radiimamomleko.com gizmlekarstva@siol.net
FENIL - National Federation of Dairy Industries	Ayala, 10 - 1º izda E - 28001 Madrid +34 91 576 21 00 +34 91 576 21 17 http://www.fenil.org info@fenil.org
Swedish Dairy Association	P.O. Box 210 S - 101 24 Stockholm

		+46 771 19 19 00 +46 8 218 363 http://www.svenskmjolk.se info@svenskmjolk.se
	Dairy UK	93 Baker Street UK - London W1U 6QQ +44 207 467 26 02 +44 207 487 47 34 http://www.dairyUk.org info@dairyUK.org

11.-ATTACHMENT

11.1 Deliverable review report

Date		Venue	
Reviewer			
Company			

11.2 Technical result of the Deliverable

Deliverable covers the topic specified in the title					
Yes		Partly		No	

Technical contents are relevant to ECO-CEMENT and to the WPs					
Yes		Partly		No	

Presented results in the Deliverable are of high value					
Yes		Partly		No	

Technical sound of the Deliverable					
Good		Regular		Bad	

Described work in the Deliverable follows a clear methodology					
Good		Regular		Bad	

Please add your comments on the content and the technical results of the Deliverable. Please comment the problems, if any.

Comments :

11.3 Length, structure and presentation of the Deliverable

Adequate length of the Deliverable					
Good		Regular		Bad	

Deliverable organization is appropriate					
Good		Regular		Bad	

Presentation of the Deliverable clear and concise					
Good		Regular		Bad	

Please add your comments on the length, the structure and the presentation.

Comments :

11.4 Rating for the Deliverable

Please provide a rating for this Deliverable from 5 (excellent) to 1 (very poor): ____

Deliverable is							
Accepted		Accepted with revisions		Rejected unless modified as suggested		Rejected	

Comments :