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UNIVERSITÉ DE MONTRÉAL

LACTIC ACID PRODUCTION FROM AGRIBUSINESS WASTE STARCH FERMENTATION WITH LACTOBACILLUS AMYLOPHILUS AND ITS CRADLE-TO-GATE LIFE CYCLE ASSESSMENT AS A PRECURSOR TO POLY-$_L$-LACTIDE

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Ce mémoire intitulé:

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présenté par : HARBEC Andréanne

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DEDICATION

To my parents, for their unconditional support

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RÉSUMÉ

Jusqu'à présent, la production industrielle du polylactide (PLA) en Amérique du Nord repose essentiellement sur la culture du maïs, qui est une ressource renouvelable. Toutefois, certaines problématiques, tels que la surexploitation des terres arables, la hausse du prix des céréales et l'usage abusif de fertilisants chimiques, ont été soulevées face à la production du maïs, surtout en ce qui a trait à son utilisation autre qu'alimentaire. Les déchets agroalimentaires seraient une alternative attrayante au maïs, de par leur riche contenu en carbohydrates, leur composition stable et leur disponibilité. Ainsi, ils représentent un potentiel de valorisation de déchets pour la synthèse du polylactide. Les eaux usées de l'industrie de transformation de la pomme de terre font partie de cette alternative. Durant la coupe des pommes de terre, un débit d'eau enlève l'excès d'amidon. Cet amidon peut être récupéré par centrifugation.

NatureWorks LLC, une division de Cargill, possède la seule usine à grande échelle pour la production commerciale de PLA. L'acide lactique produit par Cargill est obtenu par la fermentation de dextrose ($_{D}$-glucose) provenant de l'hydrolyse d'amidon de maïs. L'utilisation d'une bacteria lactique ayant des propriétés amylolytiques, c'est-à-dire pouvant hydrolyser elle-même l'amidon, permettrait de réduire les étapes de production du polylactide. *Lactobacillus amylophilus*, qui est homofermentaire, amylolytique et non-pathogénique, a été ciblé comme candidate potentielle pour la production d'acide lactique par fermentation.

Une analyse du cycle de vie (ACV) comparative de type berceau à la porte de l'usine (« cradle-to-gate ») a été réalisée afin d'évaluer les possibles bénéfices environnementaux qui pourraient découler de la production du polylactide via la fermentation directe d'un résidu d'amidon, récupéré des eaux usées d'une usine de production de croustilles, avec *L. amylophilus*. Cette étude environnementale a été menée en comparant les impacts de cette méthode de production de l'acide lactique à celle d'un procédé conventionnel à partir de dextrose de maïs. Préliminairement à cette étude, des expérimentations en laboratoire ont été réalisées et ont démontré la faisabilité microbiologique de produire de l'acide lactique avec ce déchet et *L. amylophilus*. Les pourcentages d'acide lactique obtenus par la fermentation d'un milieu synthétique de glucose,

d'amidon de patate commercial et de résidu d'amidon ont été comparés. L'analyse des résultats de l'ACV ont démontré que le polylactide produit à partir de ce résidu de la pomme de terre, et dont le procédé de production d'acide lactique a subi entre autre une optimisation adéquate de sa consommation énergétique pourrait avoir des impacts environnementaux au moins équivalents à ceux du polylactide produit via le procédé conventionnel. Cette analyse du cycle de vie a permis de soulever que l'utilisation de vapeur lors de la concentration de l'acide lactique est un processus qui contribue de façon considérable aux impacts sur les ressources et les changements climatiques du polylactide. Ainsi, une fermentation menant à une concentration plus faible d'acide lactique requière une optimisation énergétique accrue. Le peu de publications existantes sur *L. amylophilus* et l'inaccessibilité aux données industrielles furent partie des contraintes rencontrées pour ce projet.

ABSTRACT

Up to now, the industrial synthesis of polylactic acid (PLA) in North America relies essentially on the production of corn, which is a renewable resource. However, problems such as over exploitation of fertile soils, the increase of cereal price and the abusive use of chemical fertilizers, have been raised for this crop production, especially for its non-food utilization. Agri-food wastes show a promising alternative with their rich carbohydrate content, relatively stable composition and availability. Therefore, they represent an appealing waste valorization target for an eventual PLA production. Potato wastewater is one of these. During the slicing process in potato transformation, water is used to remove the excess of starch. Starch can be recovered from water after proper centrifugations.

NatureWorks LLC has the only large-scale commercial production facility of PLA and is totally owned by Cargill. Lactic acid is presently produced by Cargill with the fermentation of dextrose ($_{D}$-glucose), from the hydrolysis of corn starch. The use of an amylolytic lactic acid micro-organism could allow the direct fermentation of starch, reducing the processing steps for lactic acid synthesis. *Lactobacillus amylophilus*, which has homofermentative, amylolytic and non-pathogenic characteristics, could be an interesting micro-organism for $_{L}$-lactic acid production.

A comparative cradle-to-gate life cycle assessment (LCA) was carried out to evaluate the potential environmental benefits of producing lactic acid for polylactide usage by directly fermenting potato waste starch, recuperated from the wastewater of a potato chip facility, with *L. amylophilus*. This environmental assessment was carried out by comparing its environmental impacts to the conventional process of lactic acid fermentation of dextrose from corn. Laboratory-scale experiments were performed and have proven the microbial feasibility of producing lactic acid with *L. amylophilus* and this agribusiness residue. To do so, lactic acid concentrations obtained from synthetic media fermentations supplied with glucose, commercial potato starch or potato waste starch were compared. The LCA results have demonstrated that polylactide produced from potato waste starch could have, with proper energy and nutrient concentration optimization, at least more or less the same impact in most end-point categories studied. This LCA has underlined that steam utilization is a major contributor process in climate

change and resource depletion impacts and that a fermentation which leads to a more diluted final lactic acid concentration require more energy utilization improvements. The lack of publications about *L. amylophilus* and the inaccessibility to industrial data proved to be challenges in this project.

CONDENSÉ EN FRANÇAIS

NatureWorks LLC, une division de Cargill, possède la seule usine à grande échelle pour la production commerciale de polylactide (PLA). L'acide lactique produit par Cargill est obtenu par la fermentation de dextrose ($_D$-glucose) provenant de l'hydrolyse d'amidon de maïs, une ressource alimentaire. La présente étude a pour but d'évaluer les possibles bénéfices environnementaux qui pourraient découler de la production d'acide lactique, pour la synthèse du poly-$_L$-lactide (PLLA), à partir des eaux usées d'une compagnie de fabrication de croustilles. Plus précisément, cette étude environnementale a été réalisée à l'aide d'une analyse du cycle de vie (ACV) comparative de type berceau à la porte de l'usine (« cradle-to-gate »), permettant de comparer les impacts environnementaux de la fermentation directe d'un résidu d'amidon de patate avec *Lactobacillus amylophilus*, en remplacement du procédé de fermentation du dextrose provenant de l'amidon de maïs. Le résidu est de l'amidon récupéré des eaux usées d'une usine de fabrication de croustilles par centrifugation. *L. amylophilus* a été sélectionné pour réaliser les fermentations lactiques, de part ses caractéristiques amylolytiques, qui lui confèrent la propriété de synthétiser elle-même les enzymes nécessaires pour hydrolyser l'amidon, permettant de sauver les étapes de saccharification. Aussi, puisqu'elle est homofermentaire, non-pathogène et produit de l'acide $_L$-lactique. Aucune publication à ce jour n'a porté sur la fermentation d'un résidu de pomme de terre avec *L. amylophilus* ou sur une ACV portant sur la production du polylactide à partir d'un déchet. Les objectifs détaillés de cette présente étude sont présentés au chapitre 1.

Préalablement à l'analyse du cycle de vie, des expériences à l'échelle labo ont été réalisées afin de démontrer la faisabilité microbiologique de produire de l'acide lactique avec *L. amylophilus (NRRL-B4437)* et le résidu de pomme de terre. Des fermentations dans des bioréacteurs de 2L ont été menées sur une période de 3 jours avec un milieu synthétique supplémenté de 20 g/L de glucose, d'amidon de pomme de terre commercial ou de résidu d'amidon, préalablement stérilisé. L'influence de l'âge et du milieu de culture de la pré-culture ont été auparavant étudiés afin de déterminer si l'induction de l'activité enzymatique pouvait avoir un impact sur les taux de production d'acide lactique. Les résultats obtenus lors des fermentations avec les trois types de milieu ont démontré qu'il n'y avait pas de différence majeure au niveau de la croissance microbienne entre les trois substrats (voir Figure 2-5). Quant à la production d'acide lactique,

cette dernière est décalée dans le temps pour les milieux amidonnés, résultant du temps supplémentaire nécessaire pour hydrolyser l'amidon (voir Figure 2-6). Entre les deux milieux amidonnés, les concentrations en acide lactique produites diffèrent, étant légèrement plus élevées dans le cas du résidu de pomme de terre. Ceci peut s'expliquer par les différentes propriétés physico-chimiques (ex. : ratio amylose/amylopectin, contenu en phosphate, etc.) de ces deux amidons aux origines distinctes, pouvant influer, entre autres, sur le processus de gélatinisation lors de la stérilisation et leur digestibilité finale. Dans le cadre de ces travaux, certains facteurs pouvant justifier un tel écart de rendement ont été soulevés, mais aucun n'a pu être formellement identifié. Toutefois, le pourcentage d'acide lactique obtenu avec le résidu démontre que ce dernier tend à rejoindre la concentration finale obtenue avec le milieu optimal, celui de glucose. Ces résultats permettent de conclure à la faisabilité microbiologique de produire de l'acide lactique avec *L. amylophilus* et le résidu d'amidon et le potentiel de valorisation de déchet qui s'en découle après optimisation des procédés de fermentation.

Suivant ces travaux en laboratoire, les diagrammes d'écoulement et les paramètres de fermentation (concentration en substrat, rendement, etc.) des deux procédés à l'échelle industrielle ont été fixés. Dans le cas du procédé de fermentation du dextrose produit à partir de maïs, l'accès à des données industrielles fut impossible, pour des raisons de confidentialité. En ce qui a trait à la fermentation directe du résidu de pomme de terre, aucun procédé industriel n'existe à l'heure actuelle. Le choix des diagrammes d'écoulement et des paramètres de fermentation ont été fixés à l'aide de la littérature et d'avis d'experts et diffèrent donc de celles en laboratoire, où des concentrations faibles en substrat ont été utilisées. Il s'agit donc, dans le cas du procédé à partir du résidu, de paramètres présumés d'un futur procédé industriel, après un certain degré d'optimisation. Le choix de ces paramètres est néanmoins demeuré conservateur, étant donné le peu d'information qui a pu être relevé de la littérature sur la fermentation d'amidon de pomme de terre avec *L. amylophilus*. Dans le cas de la fermentation du dextrose de maïs, les paramètres choisis sont ceux d'une usine dite « conventionnelle ». Suivant cette pratique, le choix des unités pour la purification des deux procédés a été basé sur la dernier diagramme de procédé publié par NatureWorks (Vink, et al., 2007). Les paramètres de fermentation sont présentés au Tableau 2-1 et 2-2. Les unités utilisées pour la fermentation et la

purification de l'acide lactique sont similaires pour les deux procédés et sont présentés à la Figure 2-10.

Le dextrose est produit dans une usine de « corn wet milling » (« mouture humide »), au cours duquel l'amidon de maïs est extrait des grains à l'aide d'eau sulfurée. L'amidon est par la suite hydrolysé à l'aide d'enzymes ou d'acides. Au cours de l'extraction de l'amidon, différents coproduits sont formés et utilisés entre autre pour la production de nourriture animale. Le résidu est quant à lui concentré des eaux usées par centrifugation et filtration sous vide. Il est finalement asséché jusqu'à 13% d'humidité, à l'aide d'un séchoir. Le milieu, composé d'extrait soluble de maïs (« corn steep liquor ») et du substrat (dextrose ou résidu) est alimenté au fermenteur. Dans le cas du résidu, le milieu est préalablement gélatinisé à l'intérieur du fermenteur à 90°C, puisque l'amidon native est non soluble et peu digestible. Suite à l'inoculation avec *L. amylophilus* dans le cas du résidu et d'une bactérie lactique non amylotytique dans le cas du dextrose, l'acide lactique est produit et neutralisé avec de l'hydroxyde de calcium afin de maintenir le pH constant. À pH 6, soit le pH de fermentation, pratiquement tout l'acide lactique est sous la forme de calcium lactate. Les étapes de purification du milieu de culture suivant sa fermentation sont présentées ci-dessous:

- Retrait de la biomasse par microfiltration
- Précipitation du gypse pour dissocier l'acide lactique du calcium, à l'aide d'acide sulfurique : $C_3H_5O_3^-Ca^+O_3^-H_5C_3 + H_2SO_4 \rightarrow CaSO_4 + 2C_3H_6O_3$
- Retrait du gypse par filtration rotative;
- Concentration de l'acide lactique à l'aide d'un évaporateur à triple effets, afin de mener à une solution d'acide lactique à 88% massique;
- Une purification finale suit, afin de retirer le contenu résiduel en eau. Elle devrait être réalisée, selon la méthode traditionnelle, par l'estérification/hydrolyse de l'acide lactique. Les impuretés restantes peuvent être enlevées par l'ultrafiltration ou à l'aide d'un traitement au carbone.

Subséquemment aux choix des diagrammes d'écoulement et des paramètres de fermentation, une analyse du cycle vie comparative a été réalisée afin de comparer les impacts environnementaux de la production d'acide lactique pour synthétiser du poly-$_L$-lactide par ces deux procédés. Le cadre de l'étude est basé sur les standards ISO 14040 et 14044. Les limites du système des deux scénarios étudiés sont présentées aux Figure 3-1 et 3-2. Le scénario « Corn » est le procédé par lequel l'acide lactique est produit par la fermentation du dextrose, provenant de l'hydrolyse de l'amidon de maïs; le scénario « PWS water », par la fermentation directe du résidu d'amidon de pomme de terre, concentré des eaux usées d'une usine de transformation de la pomme de terre. Les étapes du système pour ces deux scénarios sont la préparation du milieu, le préchauffage du milieu, la fermentation et la purification de l'acide lactique.

Le transport des matières premières et de la biomasse, de même que le traitement de cette dernière et des eaux usées font partie des limites des systèmes. Étant donné que les impacts de la purification finale suivant la concentration dans l'évaporateur devraient être similaires entre les deux procédés, ils ont donc été exclus. La polymérisation de l'acide lactique, une fois purifié, est identique pour les deux scénarios. L'unité fonctionnelle est donc est 1 kg d'acide lactique à 88% massique, excluant les impuretés finales, pour la synthèse d'un polymère biodégradable, le polylactide, utilisé pour la fabrication d'emballage alimentaire. À partir des diagrammes d'écoulement et des paramètres choisis, des bilans de masse et d'énergie ont pu être réalisés (voir Annexe E). Les flux de référence, pour les deux scénarios, ont été calculés à partir de ces bilans (voir Tableau 3-1 et 3-2). Afin de traduire ses flux de références en termes d'émissions et d'énergie primaire, la base de données Ecoinvent a été choisie. Quant au dextrose, au résidu et à la liqueur de maïs, aucun processus Ecoinvent n'existe. Des processus ont été créés pour ces flux de référence, basés sur des données industrielles et la littérature (collecte de données primaire). Dans le cas du procédé de synthèse du dextrose, des coproduits sont générés, soit du gros gluten de maïs (« corn gluten feed »), de la farine gluten de maïs (« corn gluten meal »), de l'huile de maïs (« corn oil ») et de l'extrait soluble de maïs (« corn steep liquor »). Une extension du système a été utilisée afin d'éviter l'allocation. De la farine de graines de soya (« soybean meal »), de l'huile de soya (« soybean oil ») et de l'orge (« barley ») ont été les trois produits considérés comme étant évités par la production des coproduits. Dans le cas de l'extrait soluble de maïs, les impacts qui lui sont associés sont ceux du produit qu'il évite par l'extension du

système, soit la farine de graines de soya. Tous les processus Ecoinvent représente le contexte Européen, à l'exception du maïs et des produits déplacés par l'extension du système.

La méthode d'analyse d'impact « IMPACT 2002+ » a été utilisée afin de simuler les impacts des deux scénarios étudiés et ce, par chaque étape du procédé. Les résultats au niveau des catégories de dommage (utilisation des ressources, changement climatique, écosystème et santé humaine) démontrent que le scénario « PWS water », à son design actuel, n'est pas avantageux par rapport au procédé conventionnel du scénario « Corn ». En effet, comme démontré sur les Figures 3-4 à 3-7, les impacts totaux associés à ce scénario sont toujours plus élevés. Les étapes de préchauffage du milieu et de purification du scénario « PWS water » ont au moins 70% plus d'impacts que le scénario « Corn » pour chaque catégorie. En effet, le scénario « PWS water » requiert une plus grande quantité de vapeur, puisque le milieu doit être gélatinisé et l'acide lactique est plus dilué à la sortie du fermenteur. Cette consommation plus élevée de vapeur est particulièrement néfaste sur l'utilisation des ressources et les changements climatiques. La production de vapeur nécessite la combustion de gaz naturel et d'huiles lourdes, menant à des émissions de dioxyde de carbone (CO_2) et de méthane (CH_4). De plus, de façon surprenante, la préparation du milieu à partir du résidu a plus d'impact sur l'utilisation des ressources que celui du scénario « Corn » (16 MJ/kg d'acide lactique versus 11 MJ primaire/kg d'acide lactique), bien que la production du résidu, par concentration des eaux usées, soit un procédé beaucoup plus simple que celui du « corn wet milling ». En regardant de plus près, on constate que la concentration du résidu est énergivore au niveau du séchoir et même au niveau de la consommation électrique. Constatant ces faits, deux variantes au scénario « PWS water » ont été étudiées.

La première, appelée « PWS water optimized », est celle où des optimisations d'énergie ont été réalisées au niveau de la gélatinisation et de la concentration du résidu. La gélatinisation, précédemment réalisée en batch dans le fermenteur, est effectuée dans une cuve séparée, de façon continue durant le remplissage du fermenteur. Selon le même principe d'une stérilisation en continue, le courant sortant de la cuve préchauffe le courant d'entrée, via un échangeur de chaleur externe. Du côté de la concentration du résidu, des gaz de combustion sont récupérés dans l'usine

de transformation et permettent d'éviter l'utilisation de gaz naturel pour le séchoir. Dans le cas de la deuxième variante, appelée « PWS water optimized + 6 effects », trois effets sont aussi ajoutés à l'évaporateur du scenario « PWS water ». En passant de la variante « PWS water optimized » à cette dernière, on constate une baisse des impacts pour les étapes de préparation du milieu, de son prétraitement et de la purification (voir Figure 3-15 à 3-18). Pour toutes les catégories d'impacts, les impacts totaux entre les deux scénarios se sont rapprochés de façon substantielle (~ 0-30% de différence), à l'exception du dommage à l'écosystème, qui est environ 60% plus élevé dans le cas du scénario « PWS water optimized + 6 effects ». Ceci résulte majoritairement de l'intrant de liqueur de maïs pour la préparation de son milieu. Puisque les deux scénarios considèrent la même concentration de ce nutriment dans le milieu de culture, la quantité entrante de liqueur de maïs par kg d'acide lactique est supérieure pour le scénario « PWS water optimized + 6 effects ». En effet, la concentration d'acide lactique produite y est plus diluée. Puisqu'il a été considéré que la liqueur de maïs a des impacts associés à la farine de graines de soya, le produit qu'il déplace par l'extension du système, la liqueur de maïs a les mêmes impacts sur l'occupation des terres que cette farine, qui est particulièrement élevée, du moins comparativement au maïs. Le résumé des impacts de ces deux scénarios sont présentés au Tableau 3-11.

En plus de relever le peu de littérature portant sur l'espèce *L. amylophilus* et l'inaccessibilité aux données industrielles, cette étude a permis de constater le comportement complexe de l'amidon et la nécessité d'une étude approfondie de ses propriétés physicochimiques afin d'avoir la meilleure digestibilité qui puisse lui être associée. Pour évaluer le réel potentiel des cultures de *L. amylophilus* pour des applications industrielles, les conditions de pré-culture et leurs génétiques devraient être améliorées afin de connaître les rendements maximaux possibles.

Cette analyse du cycle de vie a permis de constater que la consommation de vapeur est un processus ayant une contribution majeure au niveau des impacts. Par des optimisations d'énergie appropriées, par l'ajout d'effets à l'évaporateur par exemple, une fermentation menant à une concentration plus faible d'acide lactique peut voir ses consommations d'énergie réduite à un niveau similaire que ceux d'un procédé conventionnel. La concentration en nutriments devrait être aussi optimisée de façon à minimiser son apport, tout en conservant des taux de production

intéressants. Il a aussi été relevé que l'apport d'énergie nécessaire pour concentrer l'amidon est sensible à la consommation électrique et une attention particulière devrait y être portée afin d'améliorer l'écobilan du résidu. La fermentation d'un milieu plus concentré en résidu pourrait aussi être envisagée, et devrait résulter en une concentration en acide lactique plus élevée. Toutefois, elle accroît la viscosité et les rendements en acide lactique associés sont rapidement réduits avec *L. amylophilus* selon la littérature actuelle. Il serait donc nécessaire d'évaluer l'optimum d'une concentration plus élevée d'acide lactique, associé à un rendement d'utilisation du résidu et des nutriments moindres. Pour y parvenir, des méthodes plus adaptées pour la gélatinisation, permettant un cisaillement élevé des granules, permettraient d'abaisser les coûts énergétiques associés à ce prétraitement et de diminuer la viscosité résultante.

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A: area (m^2)

aq: aqueous phase

CFU: Colony Forming Unit

Cp: heat capacity (J/K·mol or J/K·kg)

CSL: corn steep liquor

D: impeller diameter (m)

DSC: Differential Scanning Calorimetry

FCC: Food Chemicals Codex

H: enthalpy (J/mol or J/kg)

H_f: enthalpy of formation (J/mol or J/kg)

H^o_f: enthalpy of formation, standard (J/mol or J/kg)

HPLC: High Performance Liquid Chromatography

l: liquid phase

m: mass (kg)

M: molecular weight

N: rotation speed of mixing impeller (RPM)

N_p: power number

P: power (W)

q: heat transfer rate (W)

q_i: heat transfer rate at effect "i"

$q_{1\ effect}$: heat transfer rate of a one effect evaporator

PWS: potato waste starch

Re: Reynolds number

s: solid phase

U: global heat transfer ($W/m^2 \cdot °C$)

USP: United States Pharmacopeial

V: volume (m^3)

x_i: mass fraction of substance "i"

ρ: density (kg/m^3)

ΔT: variation of temperature (°C or K)

μ: viscosity (Pa·s)

ν: stoichiometric coefficient

LIST OF APPENDICES

INTRODUCTION

During the recent years, the public's growing awareness for a cleaner environment has raised a lot of expectations from biopolymers as an alternative to replace petroleum-based plastics, particularly for packaging applications (Madival, et al., 2009). Indeed, their property to biodegrade is appealing for their utilization in agriculture and packaging, or as disposables (Uihlein, et al., 2008) thus preventing accumulation of wastes in the environment. Since they are produced from renewable resources, mainly protein and carbohydrate materials, such as corn, they tend to be biodegradable as micro-organisms present in the environment can produce enzymes to attack these molecules (Kijchavengkul, et al., 2008). This biodegradation leads to carbon dioxide (CO_2), water and biomass in aerobic conditions. If anaerobe conditions are met, biodegradation will lead to methane (CO_4) instead of carbon dioxide.

A lot of confusion is related to the different terms used to label these new materials, such as "biopolymers" and "biodegradable polymers". Biopolymers are made from renewable resources, and as mentioned before, are inherently biodegradable. They can be made from the polymerization of carbohydrate polymers, such as starch, or from a fermentation-based process, as it the case for polyhydroxyalkanoates (PHAs) or polylactic acids (PLAs). A biodegradable polymer is a plastic that can degrade in the presence of naturally occurring microorganisms, which means that it can also be made from non-renewable resources (Kijchavengkul, et al., 2008). In the case of a compostable polymer, it will achieve total mineralization at a high rate in composting conditions (James, et al., 2005).

PLA, which is one of the most widely used biodegradable plastics, is compostable and recognized as having comparable mechanical and physical properties to polyethylene terephthalate (PET) and polystyrene (PS). This thermoplastic is used to package short shelf life products not exposed to high humidity and temperature conditions in packaging such as blister, clamshell, trays and bottles (Greer, 2006; Madival, et al., 2009). Other possible utilizations for PLA might be, additionally, apparels, fabrics and carpets manufacturing (Vink, et al., 2003). Since PLA is totally bioresorbable (i.e.: assimilated by the human body) and biocompatible, it

has also different applications in the medical field, such as surgical sutures, controlled drug delivery system and prostheses (Auras, et al., 2004; Lunt, 1998).

Since biopolymers are made from renewable resources, products made from bio-based polymer are generally considered to be more sustainable compared to petroleum-based alternatives (L. Shen, et al., 2008). In the case of fermentation-based processes to produce polymer such as PLA, they offer intuitive benefits, such as an aqueous processing environment and nontoxic waste (Uihlein, et al., 2008). This is true without regards to their compostability, which makes them totally biodegradable in a composting facility. This property makes biopolymers also appealing for packaging devices, for which recycling might not be practicable once they have been soiled (Kale, et al., 2007). Unfortunately, those high expectations are unbalanced by some ecological disadvantages. For example, exploitation of renewable resources leads to intensive land use (Uihlein, et al., 2008). Also, in today's waste management reality, the most common method for waste disposal is landfilling. It means that a lot of biopolymer devices might end up in landfills, where aerobic degradation is not favored and so might lead to methane formation (Lou, et al., 2009), which has 7 times more impact on climate change than CO_2 (IMPACT 2002+; Jolliet et al. (2003)). PLA production relies on the fermentation of sugars into lactic acid which are for the moment solely produced from the saccharification (hydrolysis) of corn in the United States (Bohlmann, 2009). It has been also often underlined in the literature and in the media, the ethical problem of using corn, a food source, for industrial products such as PLA, and the indirect land use change (ILUC) caused by those industrial productions which rely on a crop field (Dehue, et al., 2009). The ILUC of PLA production from corn has not been quantified yet. Its raw material for its production, corn crop, requires high intensive land use and fertilizers. Heavy fertilization is essential if continuous corn cultivation is planned. Moreover, water consumption of 15-19 cubic meters of water per bushel of grains is to be expected (Blanchard, 1992).

Since the appellation "green" has been used frequently without any scientific examination, it is essential to evaluate the environmental impacts of such a product during its complete life cycle. Life Cycle Assessment (LCA) is the most widely accepted method to assess the potential environmental impacts of a product or service, through the construction of an emissions and

extraction inventory. In the case of a cradle-to-grave LCA study, this inventory covers all phases of a life cycle, from the extraction of raw materials and fuels, followed by all conversion steps, the use and finally the disposal phase. A cradle-to-gate LCA will only include the extraction and manufacturing steps (L. Shen, et al., 2008). Its principles and framework are defined through ISO standards (International Organization for Standardization): ISO 14040 and ISO 14044. ISO defines an LCA as a "compilation and evaluation of the inputs, outputs and the potential environmental impacts of a product system throughout its life cycle" (ISO, 2006a). From the inventory, an impact assessment and its interpretation can be made.

Different LCAs on PLA products were published during the last few years: James, et al. (2005), Franklin Associates (2006), Vidal, et al. (2007), Franklin Associates (2006), Krüger, et al. (2009), Martino, et al. (2006), Hakala, et al. (1997), Franklin Associates (2006), Martino, et al. (2006), OVAM (2006), Uihlein, et al. (2008). Unfortunately, none of them present an alternative process for producing PLA than the actual one, which uses simple sugars made from food resources. By assessing a modified PLA production process, LCAs could demonstrate the possible environmental benefits of modifying, for example, the carbohydrate source and the mode of fermentation. Agribusiness residues, with their rich carbohydrate content, their quite stable composition and their availability, represent an appealing valorization target for polylactic acid production. These wastes do not make any competition to human food. It would also avoid ILUC and the use of pesticides and fertilizers. Also, possible energy savings could be realized by reducing the process steps. However, there is no LCA which has attempted to study these modifications and to demonstrate the environmental advantages and disadvantages of continuing research in these directions.

CHAPTER 1

1.1 Objectives

The present study has as a primary goal to demonstrate the possible environmental benefits or disadvantages of producing poly-L-lactide (PLLA) through an innovative process by which lactic acid for its polymerization is produced from the direct fermentation of an agribusiness residue substrate, potato waste starch, with an amylolytic bacterium, *Lactobacillus amylophilus*, without the help of any chemical or enzymatic substrate pre-treatment. By doing so, a comparative cradle-to-gate LCA will be realized to demonstrate if it would be favorable, from an environmental point of view, to pursue research in this direction, since there is no existing industrial facility. To do so, this new process should not have more environmental impacts than the conventional one, which ferments sugar from corn. Different objectives will need to be realized to complete this goal. These objectives are:

- To review the existing literature on PLA;
- To demonstrate, experimentally, the feasibility on a microbiological basis of producing lactic acid from the direct fermentation of potato waste starch with *L. amylophilus*;
- To perform a comparative cradle-to-gate LCA of producing lactic acid for PLLA synthesis from corn sugar versus the direct fermentation of potato waste with *L. amylophilus*;
- To compare the potential environmental impacts of producing lactic acid for PLLA through both processes;
- To evaluate if this new process is expected to be environmentally favorable and give recommendations and potential optimizations;

As mentioned before, two modifications will be evaluated in this process: the utilization of another starchy carbohydrate source and its direct fermentation compared to its complete hydrolyzed form, dextrose. Potato waste starch can be recuperated in wastewaters of potato

transformation facilities, such as potato chip or French fries facilities. During the slicing process in potato transformation, water is used to remove the excess of starch (see Figure 1-1), to prevent the sticking of potato slices or to soften the final texture of the cooked product. This starch can be recovered after proper centrifugations and used instead of refined starch. It presently has applications in the paper industry for making glossy paper. However, many industries do not recover potato starch. In Quebec, only one potato chip company could be listed as having the proper installations for recuperating starch in wastewater.

Figure 1-1: Potato processing © Snack Brands Australia

The utilization of an amylolytic bacterium, such as *L. amylophilus*, which can directly degrade starch, would suppress all the steps needed to extract and saccharified corn starch into dextrose ($_{D}$-glucose). The utilization of bacteria with amylolytic properties might be interesting from an environmental and economical point of view. A few studies have reported lactic acid fermentations on different starch wastes with an amylolytic lactic acid micro-organism. In this study, *Lactobacillus amylophilus,* an anaerobic facultative bacterium, has been selected for the potato waste starch valorization based on its homofermentative, amylolytic and non-pathogenic characteristics. It produces $_{L}$-lactic acid, which is attracting for making poly-$_{L}$-lactide.

The methodology employed to realize these objectives are presented in the section 1.3 General methodology. The next chapter presents a literature review on PLA and its processing, with a focus on the raw material processing and their fermentation. It will also review existing LCAs on PLA material.

1.2 State-of-art

Polylactic acid (PLA), also called polylactide, is produced from lactic acid (2-hydroxypropanoic acid). Lactic acid exists in two optically active stereo- isomers: $_{L}$-lactic acid and the $_{D}$-lactic acid. Therefore, poly-L-lactic acid (PLLA) or poly-L,D-lactic acid can be produced (Lunt, 1998). PLLA is a semicrystalline polymer that exhibits high tensile strength and low elongation with high modulus (Reddy, et al., 2008). PLLA, which can be made from renewable resources, has comparable mechanical and physical properties to PET and PS, petroleum-based polymer. Since PLLA is compostable, a new market has developed for making PLLA food packaging, replacing PET-PS made ones (Madival, et al., 2009).

NatureWorks LLC company has the only large-scale commercial production facilities of PLA worldwide. Its installations, situated in Blair, Nebraska, produce 140 000 tons of PLA per year. Cargill, which owns NatureWorks, has a lactic acid facility with an annual declared capacity of 180 000 tons. Cargill produces lactic acid for NatureWorks, by the fermentation of dextrose, which is provided by a corn wet milling facility. The last published description of the NatureWorks' process show that lactic acid is purified by adding sulphuric acid to the broth. It dissociates calcium lactate, formed by neutralizing lactic acid with calcium hydroxide during fermentation. The gypsum formed is removed by filtration. Lactic acid concentration and final purification to remove impurities follow (NatureWorks LLC; Vink, et al., 2007). NatureWorks has announced in 2009 that improvements in lactic acid processing were realized (NatureWorks LLC, 2009b). These improvements are not detailed.

PLA can be synthesized by two different processes: direct condensation of lactic acid or ring-opening polymerization of the cyclic lactide dimer. The latter is the preferred route since it polymerizes PLA to higher molecular weights and is employed by NatureWorks (Drumright, et al., 2000). The two preceding major processes, the corn wet milling and the lactic acid production, are discussed in the following paragraphs. A description of a conventional corn wet milling and a literature review on lactic acid, giving a focus on lactic acid produced from agribusiness sources and amylolytic micro-organisms, are also presented. A review on LCAs treating of PLA processing will give a conclusion to this state-of-art.

Corn wet milling

Corn wet milling is the process by which starch[1] is extracted from corn, to produce different products. Corn is a widely grown cereal in the United States. In fact, it is grown in every US state. In 2008, 12.1 billion (10^9) bushels were produced in the United States (USDA, 2010a). A bushel is equal to 25.4 kg of corn (Davis, 2001). The industry of corn wet milling, which can process corn starch into corn sweeteners, starch, oil, ethanol, and animal feed, has used from 30 million bushels to as much as 85-90 million bushels per month during the 2007-2008 period. Corn can also be refined through the dry milling process. However, this way of refining is used to a lesser extent. In 2008, the amount of corn refined by this process was approximately half of the one refined by the wet milling process (O'Brien, et al., 2010).

Researchers are looking at corn as a feedstock for the synthesis of non-food products, such as ethanol and biopolymers, since several initiatives are established for the utilization of renewable resources. This has lead to higher use of corn starch/sweeteners from corn refining facilities for industrial uses (Davis, 2001). In the case of ethanol, this trend is obvious, as shown in Figure 1-2:

[1] See Appendix A - Glossary

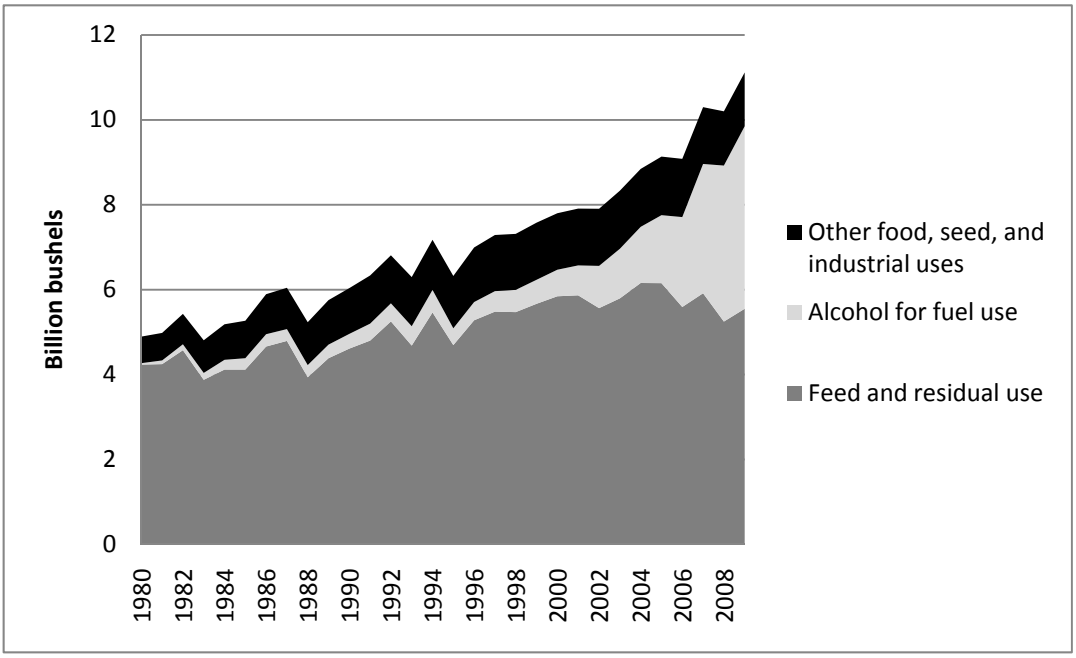

Figure 1-2: US domestic corn use (USDA, 2010b)

The corn kernel has three major parts, which are the pericarp, the endosperm and the germ. The pericarp is the outer skin of the grain, and has a role of protection. The endosperm is the energy reserve, since it contains more than 90% of starch. The germ contains mainly oil and protein (Corn Refiners Association, 2006). Yellow Dent Corn is the major specie grown in the US (Blanchard, 1992). Its typical composition is shown in Table 1-1. From this table, it can be deduced that corn contains about 62 % of starch on a wet basis.

Table 1-1: Composition of No. 2 Yellow Dent Corn (Blanchard, 1992)

	Percent (dry basis)	
	Average weight	***Standard deviation***
Starch	71.8	1.5
Protein	9.6	1.1
Oil	4.6	0.5
Crude fiber	2.9	0.5
Ash	1.4	0.2
Sugars	2.0	0.4
Moisture (wt % wet basis)	15.0	1.0

In the wet milling process, starch is extracted from the corn grain with the help of sulphurous water (Corn Refiners Association, 2006). This processing method uses all parts of the corn kernel, minimizing any corn lost. It is a mature and efficient process that has been employed in the United States since 1842 (Blanchard, 1992). Corn, which is normally stored in silos, is first cleaned to remove extraneous material and the grains are then transferred to large tanks containing steep water (Ramirez, et al., 2008). This warm water (120-130°F) contains small amounts of sulfur dioxide, forming a dilute sulfurous acid solution. For approximately 40 hours, the grains circulate in these tanks, in a countercurrent way, which means from the oldest to the newest steeps (BeMiller, et al., 2009; Corn Refiners Association, 2006; Ramirez, et al., 2008). This soaking softens the kernel, increases its moisture content and soluble components, mostly proteins, are extracted. At the end of the steeping, this protein-containing water, called "light-steep water", is concentrated in multi-effect evaporators (Ramirez, et al., 2008). This by-product, called "Corn Steep Liquor" or "Condensed Fermented Corn Extractives", can be sold as an animal feed supplement or as a nutrient source for biotechnological applications, such as enzyme production or other fermentation products. It contains about 50 wt% of solids and 23 wt% of

proteins. It is however not entirely sold in this form, since the major part of it is mix with other by-products to make corn gluten feed (Blanchard, 1992; Corn Refiners Association, 2006).

The hull of the softened kernel is ground by passing the grain through mills, with addition of water. This process liberates the oil-rich germ from the endosperm and produces a slurry containing the liberated germ. Since germ contains around 40 to 50% of oil, it is the lighter part of the slurry and so it can be separated by centrifugal forces (Corn Refiners Association, 2006; Ramirez, et al., 2008). Dry germ can be resold or the oil contained in it can be extracted by mechanical pressing. If so, the extracted germ can be used in the production of animal feeds, such as corn germ meal or corn gluten feed (Corn Refiners Association, 2006), and oil can be further refined to be sold.

The slurry containing now only the hull and the endosperm passes through a series of grinding and screening operations, where fibers are removed. The hull is held by the screen and goes generally into the constitution of corn gluten feed. It can also be used for food use (bran). The slurry of endosperms, containing starch and gluten, is directed into centrifugal separators, where a difference in their densities is employed to separate them. Gluten is dried and can be sold as corn gluten meal. Corn gluten meal and corn gluten feed, sold at 10 wt% of humidity, contain respectively about 60 wt% and 20 wt% of protein. The remaining starch slurry is then washed through hydrocyclones (Corn Refiners Association, 2006). Starch can then be dried and sold as unmodified corn starch. Typical starch and co-products yields on a dry basis are presented in Table 1-2:

Table 1-2: Typical yields of a corn wet milling facility (Blanchard, 1992)

Product/co-products	***Yield (/100 parts of dry corn)***
Steep liquor	6.5
Germ (with oil)	7.5
Bran	12.0
Gluten	5.6
Starch	68.0
Losses (volatiles, etc.)	0.4

Starch can be chemically, mechanically or physically modified and then dried. Starch can also be totally or partially hydrolyzed, with the help of acids or enzymes, to give a mixture of $_D$-glucose or soluble oligosaccharides and $_D$-glucose respectively. Ethanol can also be produced by fermenting starch hydrolyzates (BeMiller, et al., 2009). From one bushel, approximately 14.3 kg of starch or 15 kg of sweeteners or 10.6 liters of ethanol can be produced (NCGA, 2009).

A schematic process that resumes the corn wet milling steps and its different co-products is presented in Figure 1-3. It has been adapted from Davis (2001) and the Corn Refiners Association (2006). The percentage of germ extract and steep water in co-products is variable from one facility to another. More detailed process flowsheets are presented in BeMiller (2009) and Blanchard (1992). In the latter case, this reference book gives a complete view of the corn wet milling process and its products/co-products characteristics.

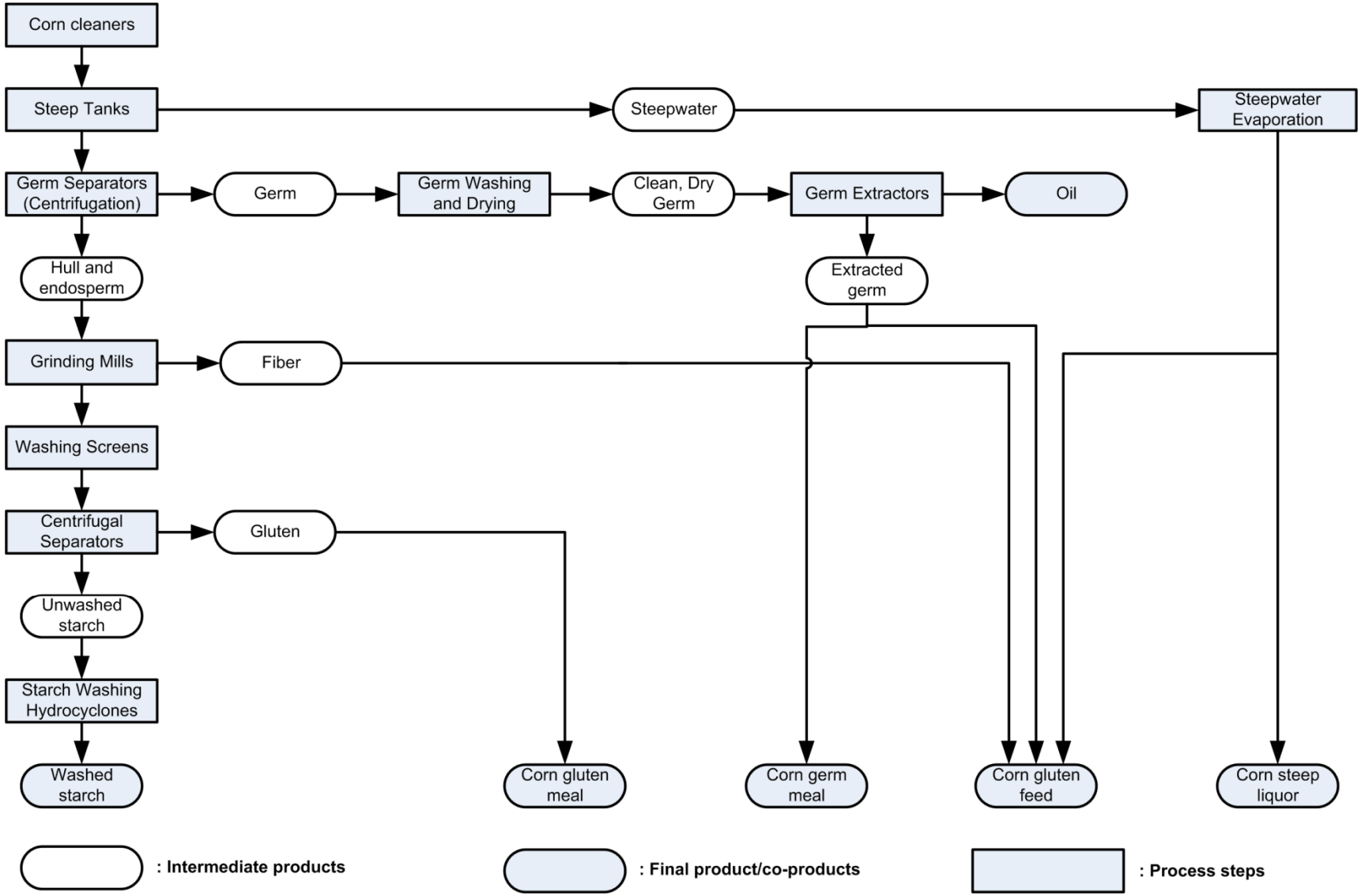

Figure 1-3: Corn wet milling block flowsheet, adapted from the Corn Refiners Association (2006) and Davis (2001)

Lactic acid production

Lactic acid is a water soluble, non-volatile, odorless and highly hygroscopic aliphatic acid. It can be produced chemically, through the production of lactonitrile as an intermediate, or by sugar fermentation of lactic acid producing micro-organisms. Fermentation is the most widespread method. The major advantages of fermentation over the chemical route are that a nearly optically pure lactic acid can be produced and renewable resources are used as raw materials. Indeed, the chemical process leads to a racemic mixture of lactic acid. Over polylactide synthesis, lactic acid has different applications in the sectors related to food preservatives, flavor enhancement, cosmetics, etc (Reddy, et al., 2008). Galactic, Purac and Cargill are major players of the North American and the European lactic acid production (Gruber, et al., 2006). Holten (1971) has published a reference book for lactic acid properties.

Lactobacillus species are well-known and widely employed lactic acid producing bacteria. Lactobacilli are Gram-positive, nonsporing, catalase-negative and anaerobe facultative bacteria. The optimum growth pH and temperature are normally respectively around 5.5-6.2 and 30°C-40°C (Vos, et al., 1984). The addition of a base during fermentation is necessary to optimize growth conditions. Depending of the species, two different patterns can be used by the bacteria for lactic acid production. The homofermentative lactobacilli utilize the EMP pathway of glucose metabolism and will produce lactic acid as a by-product. One mole of glucose will lead to 2 moles of lactic acid as shown below:

$$C_6H_{12}O_6 \xrightarrow{fermentation} 2C_3H_6O_3 + biomass$$

Some major homofermentative Lactobacilli used are *Lactobacillus delbrueckii*, *L. helveticus* and *L. casei* (John, et al., 2007). In the case of heterofermentative ones, the pentose pathway metabolism will be used, leading to the formation of lactic acid, but also other products, like ethanol, carbon dioxide and acetic acid. In few particular cases, such as carbohydrate limitation, a homofermentative lactobacilli may act as a heterofermentative one. Another characteristic of

lactobacilli is their differing ability to produce $_{L}$-lactic acid, $_{D}$-lactic acid or $_{D,L}$-lactic acid. Homofermentative lactic acid bacteria are commonly used for industrial production (Vaidya, et al., 2005). Taxonomic and properties outline of Lactobacilli can be found in Bergey's manual of systematic bacteriology.

Other genius of lactic acid bacteria are *Streptococcus*, *Leuconostoc* and *Euterococcus* (John, et al., 2007). Another well-known micro-organism capable of producing lactic acid is *Rhizopus* sp. This yeast, as a heterofermentative bacteria, produces lactic acid and other products. *Rhizopus* produces $_{L}$-lactic acid enantiomer. Oxygen has to be provided to the medium since it is an aerobic micro-organism. Adequate agitation is essential to optimize mass transfer, by dispersing oxygen and reducing the cellular aggregation and the bubble gas size. One advantage of *Rhizopus* over *Lactobacilli* is its lower nutrients requirement. However, lower lactic acid yields are normally achieved (Gruber, et al., 2006; John, et al., 2009).

Different carbohydrate feedstock might be used for lactic acid fermentation. Refined sugars have been widely used in the past, but, due to their high price, are replaced by less expensive sources. It is well known that the price of raw material remains a significant manufacturing cost. Residues and products from the agribusiness and forestry industry are cheaper sources of carbohydrates. Molasses, whey, sugarcane bagasse and starch from different plants, such as potato, corn or wheat, are sugar-containing materials interesting for lactic acid production. Since only sugar in its simplest form, glucose, can be metabolized by most micro-organisms, a proper treatment may be needed prior to fermentation to saccharify the polysaccharides to glucose. In fact, lactic acid bacteria are usually deficient in cellulolytic and amylolytic characters (John, et al., 2007), which prevents the bacteria from producing its own enzymes for hydrolyzing starch or cellulose.

In the case of starch, most species of lactobacilli cannot ferment it directly, since they do not produce the necessary amylases to convert this polysaccharide to dextrose. This is the case for *L. delbrueckii* and *L. casei*, largely employed Lactobacilli. An acid or enzymatic hydrolysis has to be performed on starch (John, et al., 2007). Steps to convert starch into glucose with enzymes are

typically gelatinization[2] and liquefaction, which are both carried out at high temperatures (90 - 130ºC) for 15 min., followed by saccharification to glucose (Reddy, et al., 2008). As mentioned before, Cargill is using this mode of production since it ferments glucose from corn starch.

Since saccharification follows a substrate inhibition kinetic model, high concentration of glucose will slow down hydrolysis (Anuradha, et al., 1999). It has been widely recommended in literature to do a simultaneous saccharification and fermentation (SSF) to minimize inhibition by glucose. Indeed, if saccharification is the limiting step, glucose is metabolized as fermentation proceeds and it will not accumulate in the culture medium. It reduces also the production cost for biomass hydrolysis (John, et al., 2009). A prior study of the enzyme and bacteria kinetics has to be performed to ensure successful realization. Anuradha (1999) is presenting a kinetic model for simultaneous saccharification and fermentation of starch by *L. delbrueckii*.

Another possibility is the utilization of an amylolytic lactic acid bacterium. Since an amylolytic bacterium produces its own amylases to hydrolyze starch, the liquefaction and saccharification steps are unnecessary. Gelatinization is however normally performed since it improves the efficiency of enzymatic hydrolysis (John, et al., 2007). The major advantage of using an amylolytic bacterium is the reduced manufacturing and operating cost related to enzymes and hydrolysis of starch. However, these possible benefits might be offset, among others, by lower production rates. Examples of amylolytic Lactobacilli are *L. amylophilus*, *L. amylovorus*, *L. plantarum* and *L. manihotivorans*. *L. amylophilus* is an appealing amylolytic homofermentative bacterium, because it synthesizes only $_{L}$-lactic, without any gas production. It is also a none pathogenic micro-organism. Different strains of *L. amylophilus* were used in literature: *L. amylophilus* GV6 (Altaf, et al., 2005; Altaf,Naveena, et al., 2007; Altaf, et al., 2006; Altaf,Venkateshwar, et al., 2007; Naveena, Altaf, et al., 2005; Naveena, et al., 2004; Naveena,Altaf, et al., 2005; Naveena, et al., 2003; Reddy, et al., 2008; Vishnu, et al., 2006), JCM 1125 (Yokota, et al., 1998; Yumoto, et al., 1995), NCIB11546 (Fitzsimons, et al., 1994) and

[2] See Appendix A -Glossary

NRRL B-4437 (Pierre Mercier, 1990; P. Mercier, et al., 1992; Pompeyo, et al., 1993) Yokota *et al.* (1998) has produced at high substrate concentration (57 g/L of potato starch) approximately 33 g/L of lactic acid. Improvement of nutrient sources has enabled the production of about 55 g/L of lactic acid from a 84 g/L medium of potato starch. Vishnu *et al.* (2002) have fermented different types of starch *with L. amylophilus GV6*. This bacteria, which produces amylase but also amylopullulanase, could produce 32 g/L and 32.6 g/L of lactic acid from, respectively, 40 g/L of potato starch and corn starch (Vishnu, et al., 2002). Five publications on *L. amylophilus GV6* are unfortunately repetitive, since they present selection of nitrogen component, mainly yeast cells and lentils, and their concentration optimization through a Plackett-Burman design or a response-surface methodology.

Over the concern of using renewable resources, there is also a desire of using waste instead of foodstuff. A few publications have discussed the fermentation of kitchen wastes (Sakai, et al., 2004; Wang,Sun, et al., 2005; Wang, Wang, et al., 2005). Agribusiness wastes, with their high production volume and their constant composition over time, are appealing substrate for lactic acid production over kitchen wastes. Some studies were performed with whey and cellulosic residue, such as corn cobs. In the first case, the major disadvantage is its diluted form and the cost associated to its concentration (Vaidya, et al., 2005). In the case of cellulosic waste, it has to be pretreated prior to fermentation, due to the compact form of cellulose and the difficulty with which enzymes can degrade it (Jeoh, et al., 2007). In the case of starchy waste, there are different publications on the lactic acid fermentation of potato waste. Most of them used the yeast *Rhizopus*, which has amylolytic properties, to ferment, among others, cull potatoes and potato wastewater (L. Huang, et al., 2005; L. P. Huang, et al., 2005; Jin, 2006; Jin, et al., 2003; Jin, et al., 2005; Li Ping Huang, 2003; Y. Liu, et al., 2005; Oda, et al., 2002; Z. Zhang, et al., 2007). There is no publication about the fermentation of starchy waste with *L. amylophilus*.

Life cycle assessment on PLA

LCAs on PLA made bags (James, et al., 2005), films (Franklin Associates, 2006; Vidal, et al., 2007), food containers (Franklin Associates, 2006; Krüger, et al., 2009; Martino, et al., 2006), diapers (Hakala, et al., 1997) , and drinking containers (Franklin Associates, 2006; Martino, et al., 2006; OVAM, 2006; Uihlein, et al., 2008) can be found in the literature. Cradle-to-gate LCAs about PLA material are scarce. The most well-known and used are the ones published by NatureWorks. In fact, most of the LCAs previously mentioned are using NatureWorks inventories. Only the LCA of Hakala et al. (1997) and Uihlein et al. (2008) are using other data sources. However, the inventory for PLA production is not presented.

NatureWorks has published two cradle-to-gate LCA studies on their PLA production during the last two decades: Vink et al. (2003) & (2007). Their production system can be described in five major steps. First, the life cycle starts with corn growing and harvesting. After, corn is sent to a corn wet milling facility where starch is separated and converted to dextrose. Dextrose solution is sent by pipeline to the fermentation process to be converted to lactic acid. The two last steps are the conversion of purified lactic acid solution to lactide and the polymerization of lactide into polylactide polymer pellets (Vink, et al., 2007).

From the published LCAs, it is possible to notice that gross non-renewable energy use has been reduced by half from 2003 to 2006, passing from 54.1 to 27.2 MJ primary /kg PLLA. Decrease in primary energy consumption was expected since biopolymers are recent technologies and, therefore, can be more easily improved compared to mature processes. In addition to on-site process optimization, this progress has been achieved with the help of REC (“Renewable Energy Certificates”) credits purchasing (Vink, et al., 2007), which stopped in January 2009 (Vink, 2010). This evolution leads to an improvement of LCA results of PLA products with years of publishing, since there are improvements in polylactide emissions and energy consumption inventory.

Unfortunately, NatureWorks LCAs presents aggregated inventories, which makes impossible the allocation of the energy and emissions to each process steps. In fact, only a few large-scale lactic acid and PLA facilities exist in the world and, therefore, the level of confidentiality on processing is high. Thus, detailed data and method of calculations are not presented in NatureWorks LCAs, which limits the use of those ecobalances, even more if processing is the point of interest. For example, as underlined by Dornburg et al. (2006), NatureWorks LCA do not present the allocation procedure for corn wet milling by-products. It has been cited that this lack of transparency might raise a doubt against the validity of these LCAs to respond to ISO standards (Jones F., 2009).

The only other published cradle-to-gate LCA on PLA was published by SRI consulting in 2004 (Bohlmann, 2004). The primary energy consumption for PLA was similar to the one of NatureWorks in 2003 (51 MJ primary/kg PLLA). The detailed process data and methods of calculation used for the realization of this LCA are presented in an SRI report. However, access to this report is limited due to its high purchase cost. Even for lactic acid, from which polylactide is polymerized, there is no published life cycle assessment. There are however few lactic acid economic assessments from which LCA could be performed (Akerberg, et al., 2000; Cable, et al., 1971; González, et al., 2007; Tejayadi, et al., 1995). They are presenting lactic acid fermentation from whey or wheat.

This portrait is in opposition to the number of published life cycle assessments on PHA (polyhydroxyalkanotes) materials, which are biodegradable polymers produced in micro-organism cells. Numerous publications are available, giving sometimes the whole process design or a financial assessment (Akiyama, et al., 2003; Devdatt Kurdikar, et al., 2000; Gerngross, 1999; Gurieff, et al., 2007; Harding, et al., 2007; S. Kim, et al., 2005; Seungdo Kim, et al., 2008; Pietrini, et al., 2007; Zhong, et al., 2009). Some studies are even looking at waste valorization or crop management. This discrepancy is quite surprising, knowing that polylactide market is presently bigger. An effort should be put into this direction to improve literature on PLA processing and its ecobalance.

1.3 General methodology

Following the literature review, which has demonstrated the lack of data related to the synthesis of PLA and the industrial utilization of foodstuffs for lactic acid production, fermentation experiments, with *L. amylophilus*, have been done with a potato waste starch medium. The lactic acid yields were compared with the ones obtained from glucose or commercial potato starch medium. The microbial feasibility is discussed and demonstrated, by comparing the obtained lactic acid production rate with the potato waste medium and the optimal one, the glucose medium. Those experiments are described in section 2.1.

Once the feasibility has been demonstrated, the industrial process flowsheet for PLA synthesis from corn and the expected one if potato starch waste starch would be directly fermented into lactic acid were drawn. The choice of the different units is based on NatureWorks and Purac published flowsheets (Purac; Vink, et al., 2007) and accepted practices in bioengineering. The different process parameters, such as yield and substrate concentration, were set, also with the help of literature and general knowledge. Assumptions were made due to the confidentiality of processing data, and also because the industrial production of PLA from potato waste does not exist.

The next step was the goal and scope definition of the cradle-to-gate LCA. Based on the fermentation parameters and the flowsheet previously set, mass and energy balances were realized and the inventory was constructed for both processes. From this inventory, a comparative impact assessment was performed and analyzed. The LCA section is presented in Chapter 3. A final discussion on the whole project is then presented. It recalls, among other topics, the environmental profitability and recommendations for optimizing the potato waste starch process. A general conclusion is also presented.

CHAPTER 2

2.1 Laboratory experiments

This section presents the results and discussion of laboratory experiments carried out to evaluate the microbial feasibility of producing lactic acid via the direct fermentation of potato waste starch with *Lactobacillus amylophilus*.

2.1.1 Materials and methods

Microorganism propagation and conservation

Amylolytic homofermentative *L. amylophilus* (NRRL B-4437) (Nakamura, et al., 1979) was purchased from the ATCC Bioresource. Pure culture was initially propagated at 30°C in MRS broth (Difco), previously sterilized. The choice of temperature was based on ATCC growth conditions (ATCC, 2009). Sterilization during experiments has been always performed with a 10 minutes/121°C cycle, in liquid mode for liquids, gravity mode for solids, in a steam sterilizer. Suspended cells grown in MRS broth were then concentrated by centrifugation and frozen at -80ºC in sterile cryogenic vials containing a 15% w/w BHI-glycerol solution.

Media preparation

Reconstituted MRS media containing 20g/L of carbohydrate were prepared with peptone (10 g/L, Difco), meat extract (8 g/L, Oxoid), yeast extract (4 g/L, Difco), dibasic potassium phosphate (2 g/L % w/v, EM Science), sodium acetate (5 g/L, BDH), ammonium citrate (2 g/L, Fisher Chemicals), magnesium sulfate, (0.2 g/L, Laboratoire Mat), manganese sulfate (0.05 g/L, Spectrum Quality Product), polysorbate 80 (1 g/L, Sigma Aldrich) and glucose (20 g/L Sigma Aldrich) or commercial potato starch (20 g/L, Sigma Aldrich, see Figure 2-1) or potato waste starch (20 g/L, anonymous industrial source, see Figure 2-1), mixed with 1 liter of deionized

water. Once the medium has been sterilized, magnetic agitation was kept constant during the cooling of starchy medium to minimize retrogradation[3] of starch.

Humidity content of potato waste starch was evaluated by drying samples at 130°C for 90 min., following the International Starch Institute ISI 01-1e method (International Starch Institute, 1999). Potato waste starch was frozen at -20°C until its use. On a dry weight basis, potato waste starch was considered as being pure, since impurities are expected to be very low for this kind of waste (less than 1%) (Cheatham, 2010).

Fermentation

Fresh inocula for fermentation (preculture) were prepared in sterile tubes containing MRS broth by adding a thawed cell suspension at 2% and incubating the tubes at 30ºC, for 24h. Nitrogen was injected in tubes prior to incubation to flush the headspace. During preculture growth, tubes were agitated on a rotary shaker (Large Labquake® Shaker, Double deck trays, Thermo Scientific) to maintain cell dispersion and minimize turbulence.

Fermentations were performed in BioFermentors (New Brunswick, see Figure 2-2), with a 2.5 L vessel and a Bioflo3000 system control. Two liters of media were inoculated with a 24h-glucose preculture of *Lactobacillus amylophilus* at 1%. Fermentations were carried out at 30°C at an initial pH of 6.1 ± 0.1 under pH control at 5.5 ± 0.1 with a 3M solution KOH/NH4OH 5:1 v/v. Choice of pH condition is based on Mercier et al. (1992) and Pompeyo et al. (1993). Agitation was carried out with a double turbine stirrer running at 50 RPM for glucose and commercial potato starch and 100 RPM for potato waste starch due to higher viscosity. Sampling was done every 24h for microbial analysis. Part of samples was also frozen at -20ºC for further chemical analysis. Fermentations were performed following a random planning, in 3 assays in the case of glucose, 4 in the case of commercial starch medium and 5 assays in the case of potato waste

[3] See Appendix A - Glossary

starch. The number of assays was chosen in function of the heterogeneity of the different medium. Potential contamination was checked with Petris and microscope observations.

Figure 2-1: Commercial potato starch (left) and potato waste starch (right)

Figure 2-2: Fermentation set-up

Effect of preculture

The effect of preculture has been investigated on commercial potato starch fermentations inoculated with a 24h preculture grown on a glucose MRS broth (Difco) or grown on a commercial potato starch reconstituted MRS medium. These experiments were realized in 2 assays. The initial pH was not adjusted (pH = 6.5) and controlled at 5.5. The age of the inoculum has also been taken in consideration (24h-48h). Microbial counts and pH measurements of precultures incubated during a period of up to 72 hours has been previously performed, in 3 assays.

Microbiological analysis

Serial dilutions were done in 0.1% peptone water (Difco). Microbial counts were numerated on MRS agar supplemented with X-Gal (Bioshop), in duplicates. MRS agar was prepared with MRS broth (Difco), to which 12g/L of agar (Difco) and 0.6 mL/L of a X-Gal solution is added after sterilization. The X-Gal solution is prepared by diluting the X-Gal at 10% (w/v) in DMSO (dimethyl sulphoxide) and sterilizing the solution by filtration on a 0.45 µm filter syringe. Final X-Gal concentration in the MRS broth is 0.006%. Incubation was done in an anaerobic chamber at 30°C, and counts were performed when maximum growth was observed (approximately after 3-4 days of incubation).

Chemical analysis

Samples were centrifuged at 4500 RPM for 10 min. at 4°C. 4 mL of supernatant was removed and diluted 1:2 with 4 mL of mobile phase, H2SO4 0.01N, with the help of a vortex mixer. The liquid was then filtrated on a 0.45 µm filter. The filtrated liquid was then subsequently separated on a “Sep-Pak C18 plus” column (Waters), previously conditioned with, successively, 8 mL of methanol and deionized water. The sample was finally filtrated with a 0.22 µm. The clarified liquid was transferred in HPLC vials and lactic acid and glucose were analyzed on HPLC (Waters), with an Aminex HPX-87H column.

2.1.2 Results and discussion

Effect of preculture

Growth and pH measurements have been previously carried out on precultures, every 24 hours. Results are presented in Appendix B. From these results, it has been decided that 24 hours preculture should be the optimum incubation time tested, since growth was in its exponential phase. Experiments to evaluate the effect of the 24h-preculture medium type (MRS glucose versus reconstituted MRS of commercial potato starch) have shown that independently of the carbohydrate sources for the preparation of preculture, no significant differences were observed for growth rate (see Figure 2-3) and lactic acids yields (see Figure 2-4) of commercial potato starch fermentations.

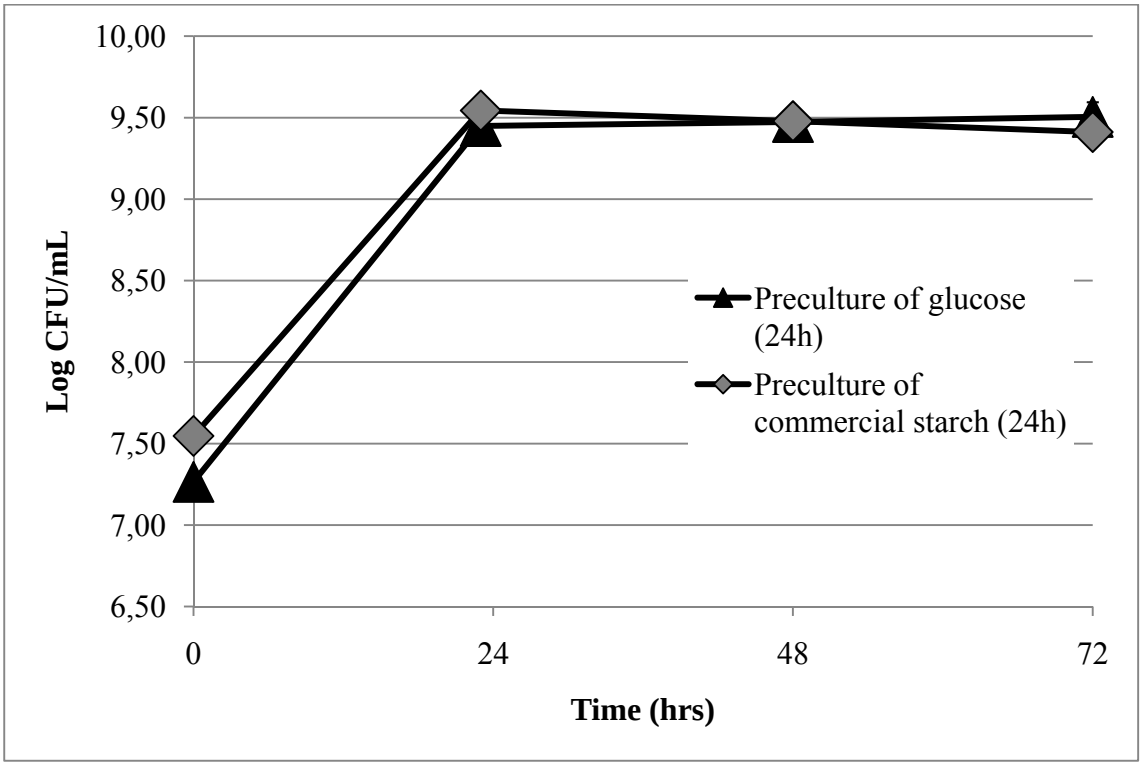

Figure 2-3: Growth in commercial potato starch fermentations as a function of preculture type

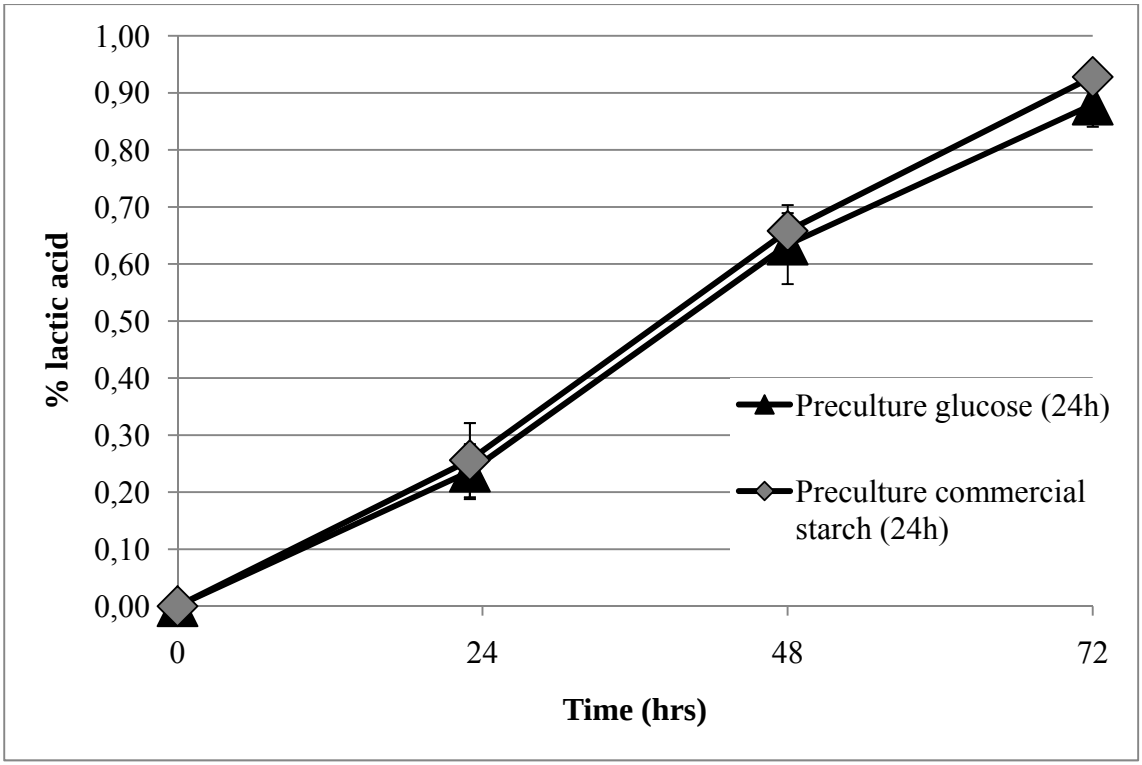

Figure 2-4: Percentage of lactic acid produced by commercial potato starch fermentations as function of preculture type

The age of the inoculum has also been evaluated (1 assay only). A 48h-preculture made of commercial potato starch had no effect on lactic acid yields (data not shown). Following this investigation, it was decided to do the next fermentations with a 24h-preculture of MRS glucose.

Growth on glucose, commercial starch and starch waste fermentation

As reported in Figure 2-5, the growth on the different media are similar. The growth on starch coming from wastewater presents a slightly higher rate and maximum. The growth could have been influenced by trace levels of proteins, lipids, phosphates, for examples, which can be variable between starches of different origins and may have favored growth in the case of potato waste starch fermentation. A nitrogen evaluation with a LECO Nitrogen / protein Determinator FP-428 (LECO Corporation) has demonstrated that the protein content was negligible in potato waste starch. Other impurity levels were not evaluated. Nevertheless, this growth difference was

not considered as significant. Independently of the media, the maximal growth is reached during the first 24 hours of fermentation.

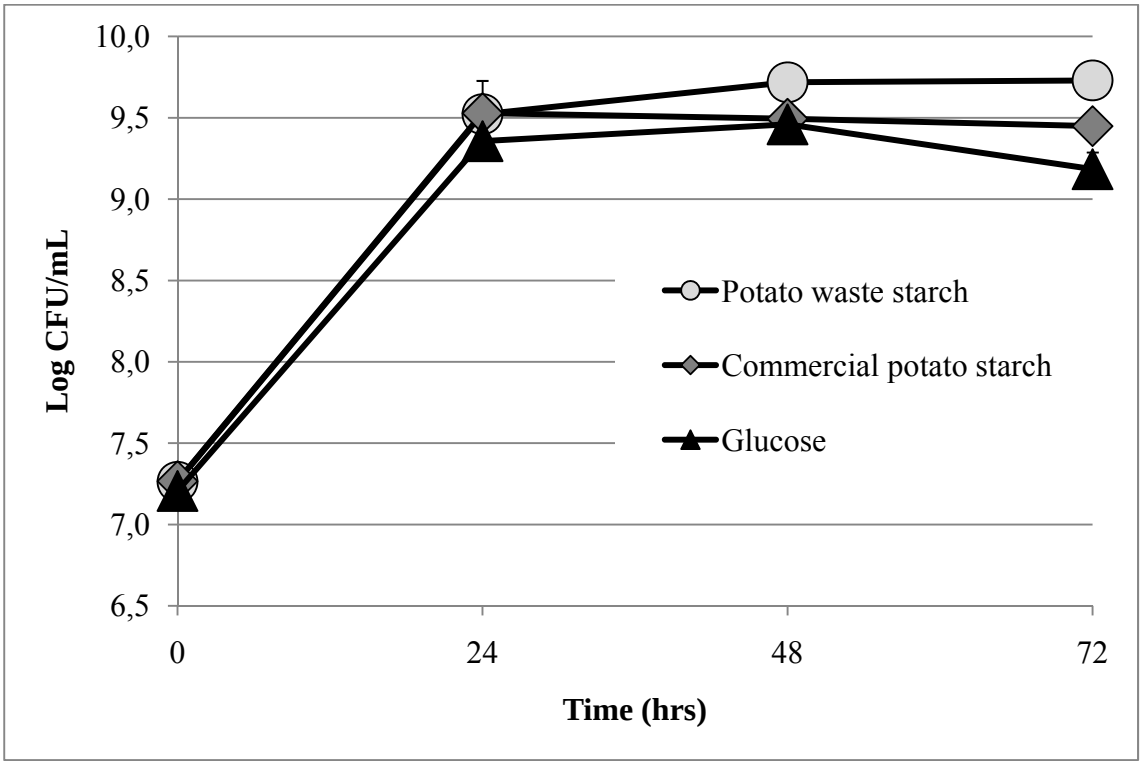

Figure 2-5: Growth as a function of fermentation time for different MRS reconstituted medium

Lactic acid production from glucose, commercial starch and starch waste fermentation

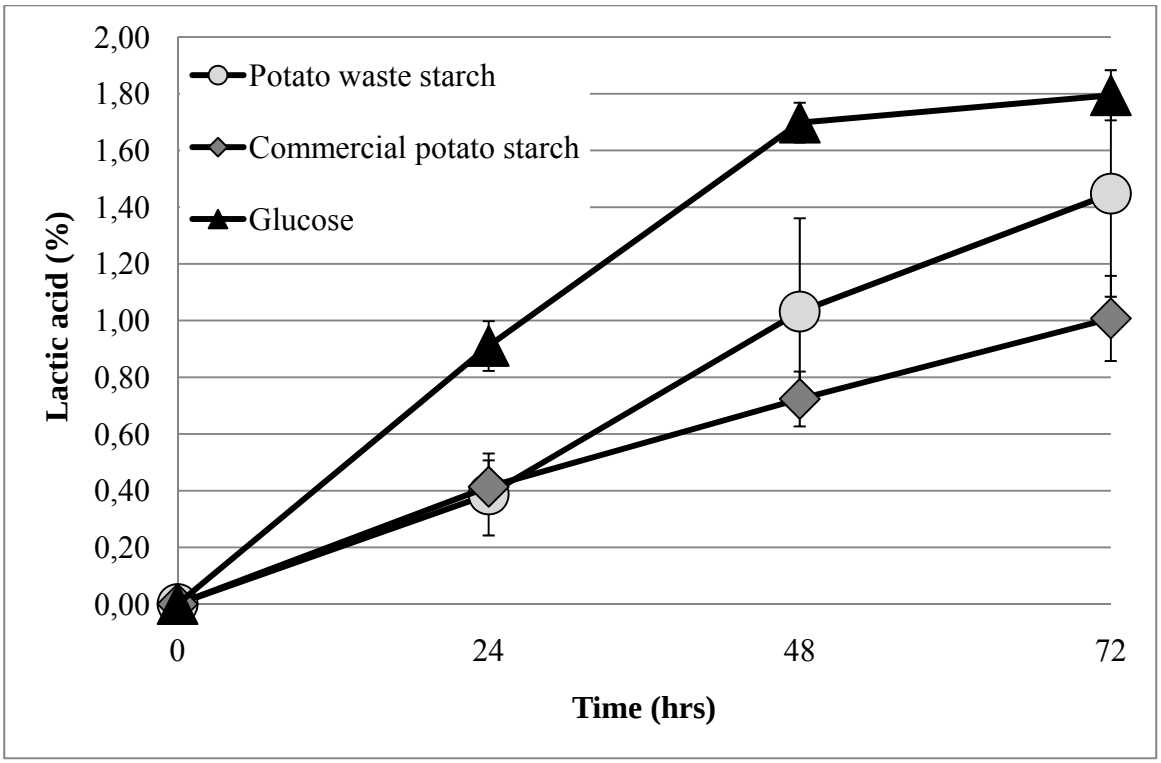

Figure 2-6: Percentage of lactic acid as a function of fermentation time for different MRS reconstituted medium

With regards to the lactic acid production, the metabolism is clearly dependant of the fermentation medium. As shown in Figure 2-6, the lactic acid production is faster and it reaches a higher final concentration when fermentation is conducted on a glucose rather than on a starch medium. This has to be expected since starch hydrolysis takes longer than direct assimilation of a single carbohydrate like glucose. Moreover, the maximal lactic acid concentration (1.79%) is almost reached in the first 48 hours and stays stable as microbial growth enters into a decline phase (see Figure 2-5). This behavior is mainly linked to the complete removal of glucose, as it was observed by HPLC results (data not shown). For the starchy media, the lactic acid production has not reached its highest level within 48 hours, thus suggesting that the latter one is not in a limiting concentration. Both starch forms (commercial and waste) show a different profile resulting in a slower lactic acid production. Commercial potato starch shows the smallest lactic

acid yield. Starch coming from wastewater allows slightly lower levels of acid after 72 hours as compared to those obtained on glucose.

For both starch substrates, the glucose concentration during fermentation was negligible, which suggests that the hydrolysis of starch, the enzyme production or its mass transfer can be the limiting step. The whole process of lactic acid production on a micro point of view can be summarized in 8 steps, depicted in Figure 2-7:

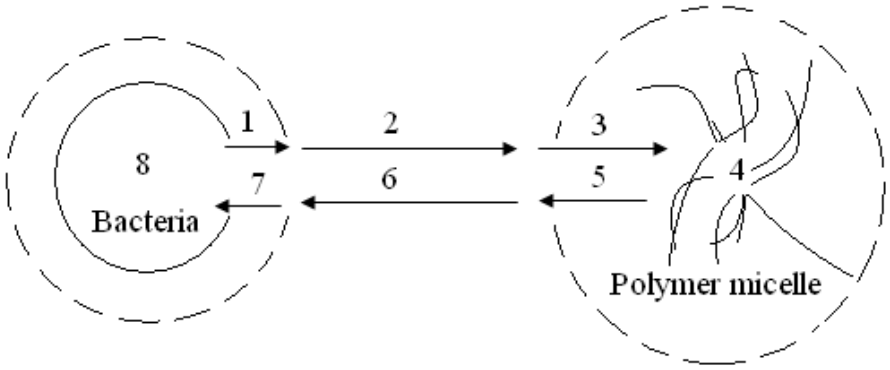

Figure 2-7: Steps in lactic acid production

1: Enzyme production diffusion; 2: Enzyme convective mass transfer; 3: Enzyme diffusion; 4: Starch hydrolysis; 5: Glucose diffusion; 6: Glucose convective mass transfer; 7: Glucose diffusion; 8: Glucose assimilation/enzyme production

The enzyme production rate for the potato waste starch media may have been influenced by the presence of impurities as mentioned previously for the growth. However, the interesting discrepancy between commercial and waste starch lactic acid yields should be mainly related to the different rate of starch hydrolysis, which relies on the different starch physicochemical properties. Many factors, such as, for examples, type of cultivar, the cultivation method and the method of isolation may influence the composition (ex.: ratio of amylose/amylopectin, phosphorus content) and the structure (ex.: crystallinity, porosity), therefore, among others, the thermal behavior (Q. Liu, et al., 2002; Qiang Liu, et al., 2007) and the digestibility of a starch

solution (Tester, et al., 2004). Since starches used in these experiments were from different origins, they may have different behaviors upon sterilization/cooling and variable final digestibility.

Microscopic observation of a small medium volume (100 mL) shows some extent of gelatinization following sterilization (see Figure 2-8).

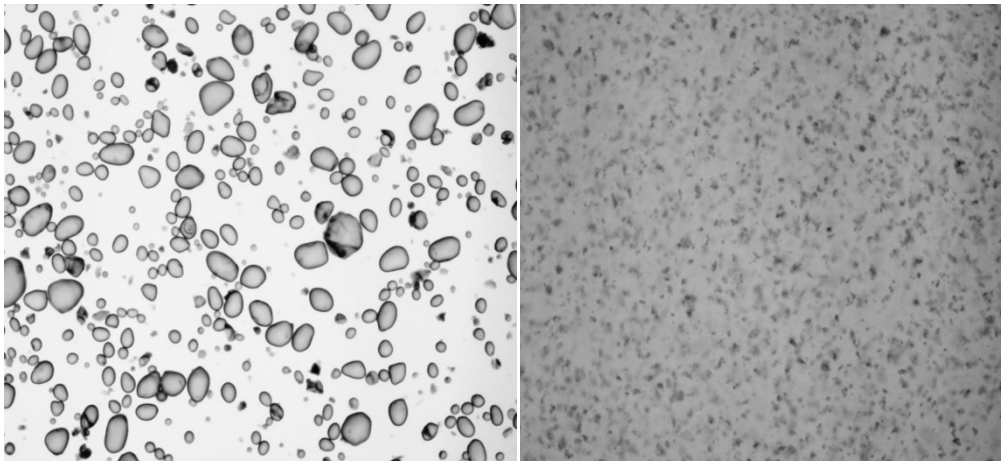

Figure 2-8: Optical microscope observation of iodine stained commercial starch medium (100 mL); Before (left) and after sterilization (right)

As reported by others, gelatinization leads to the swelling of starch granules (see Appendix A - Glossary). Further heating may lead to the release of starch polymers in the medium and to the lost of granule integrity. Gelatinization, which disrupts crystallinity of starch and dissolves polymers chains, is known for improving the binding capacities of amylase and therefore increases the hydrolysis efficiency of enzymes (Tester, et al., 2004). Higher the degree of gelatinization, higher should be the starch digestibility. In laboratory, gelatinization was performed during sterilization, in excess of water, which corresponds to a water concentration of more than about 70% w/w (Qiang, 2009).

Our results suggest that the degree of digestibility reached as a result of sterilization is higher with waste starch compared to the commercial one. Furthermore, greater viscosity has been visually observed for sterilized waste starch medium. In fact, a higher degree of solubility was noticed for gelatinized waste starch compared to commercial starch. A white precipitate could be observed in sterilized commercial starch medium when no agitation was provided. In the case of potato waste starch, firm pieces of gel were formed during sterilization. One explanation might be the different processing methods of this substrate, leading to different water binding capacity and therefore, differences in physicochemical properties. Since potato waste starch is collected in water, it can be assumed that these granules have more affinity with water. In the case of commercial starch, they are usually treated to reduce their hydroscopic properties. Also, amylopectin is more soluble in water than amylose (Bernfeld, 2006), suggesting that the residue might have a higher content of amylopectin.

The higher solubility of potato waste starch may also be explained by the degree of gelatinization reached which facilitates polymers dissolution. The degree of gelatinization is dependant of the granules swelling capacity and the water mass transfer. Potato starch is known for its high swelling capacity, leading to high peak viscosity. It is expected that at heating temperature of 100°C, in excess of water, an homogenous potato starch solution is totally disrupted (Swinkels, 1985). The lasting white powder in sterilized commercial starch medium could be starch granules swollen to a lesser extent, resulting in lower viscosity, solubility and also digestibility. Mass transfer limitation of water due to starch precipitation during heating may have been more detrimental to commercial potato starch. As mentioned before, commercial starches are treated to reduce their hygroscopic behavior and this treatment may have reduced the water absorption capacity of its granules. Potato waste starch did not receive any treatment, but was frozen for conservation purposes. Freezing and thawing increase the granule porosity, and so improve their wetting ability (Szymonska, et al., 2005), and so gelatinization efficiency. The drying temperature has also an influence on the starch granules swelling capacity and may have been different between the processing of commercial and waste starch. Higher drying temperature increase the granules rigidity, thus reduces their swelling capacities (Malumba, et al., 2009).

Retrogradation during cooling might be a cause for gel and precipitate formation. The white precipitate of commercial starch could be polymer chains highly crystallized by this set-back process. Retrogradation is known for its negative impact on digestibility since it crystallizes polymer chains and reduces enzyme accessibility (Chung, et al., 2006; Tester, et al., 2004). It may have been more detrimental in the case of commercial starch, if it contains more amylose. Amylose chains are essentially linear and have a greater tendency to reassociate upon cooling (Thomas, et al., 1999). Phosphorus content has also a negative impact on digestibility (T. Noda, et al., 2008). As it can be noticed, numerous reasons can be raised to explain the difference in digestibility of both starch medium, and the ratio of amylose/amylopectin is one of these. However, it is impossible within the framework of these experiments' results to end up with any conclusion. Different publications are presented in the bibliography to present the complexity of starch.

In the case of potato waste starch medium, initial starch could not be sampled adequately, since pieces of gel were present at the fermentor start up. These pieces of gel, which are probably more concentrated in starch than the surrounding medium, seemed to disappear within 24 hours. Therefore, it was impossible to have similar homogeneity between assays and temperature was also difficult to control at the fermentor start up. This has lead to a reduced reproducibility of the experimental data. Standard deviation for the measurements of lactic acid content was more elevated for these fermentations. Another demonstration of this complex behavior is the gel formation during freezing. Samples, frozen at -20°C for conservation, had small pieces of gel after thawing. This was also the case for commercial starch samples, but to a lesser extent. This gel formation was probably caused by retrogradation, which can happen during storage and freezing (Ribotta, et al., 2003). Heterogeneity of samples has prevented starch analysis and so the exact initial starch concentration could not be measured. A special attention must be given to storage conditions or, otherwise, conduct the analysis immediately after the samples collection.

It was assumed that starch deposition during sterilization might lead to limited water mass transfer to starch granules but also easier reassociation between the dispersed polymer chains

during cooling. To overcome gel formation, pre-heating of the medium was performed on a heating plate, up to approximately 75°C, to partly gelatinized starch before sterilization. Around 75°C, increase in viscosity was observed, a sign that most of the granules have swollen. Sterilization was then realized, by adjusting the temperature sensor to 75°C at the beginning. The final medium was approximately 35 times more viscous than water (viscometer measurements at 30°C), without any pieces of gel. The same preparation method was used for commercial starch, at a pre-heating temperature of 70°C. The homogeneity of the medium seemed also to be improved. The viscosity was approximately 1.5 times more elevated than the one of water. Some fine tuning was performed on fermentor to adjust the speed of agitation to prevent temperature variation. Fermentation assay were realized with these homogenous media. Average final lactic acid concentration was approximately 50% lower for potato waste starch (data not shown), and lactic acid curve was out of previous experiments standard deviation. Temperature control was still problematic. This procedure for gelatinization was different, in term of speed of heating and agitation, compared to direct sterilization, and reduces standard deviation of these assays, but seems unfavorable to reach a better digestion rate. The medium rheology may also have reduced the nutrients mass transfer to the cells. A decrease in final lactic acid concentration was also observed for commercial potato starch fermentation. It was reduced by more or less 30% (data not shown).

During these assays, fermentations were also performed with spent brewer's sludge (source: industrial; anonymous), replacing the equivalent nitrogen amount of MRS constituents (peptone, meat extract and yeast extract). Nitrogen contents of MRS constituents and spent brewer's sludge were realized with the LECO Nitrogen / protein Determinator. Water content of spent brewer's sludge was measured by drying at 105°C until constant weight. After sterilization, the media had a strong smell of hops. Fermentations did not give any notable lactic acid production. Hops, which inhibit lactic acid growth (Green, 2009), might be the cause.

It was also observed during experiments that a medium sterilized without polysorbate 80 was less viscous. Surfactants are known for influencing starch thermal behavior and it was demonstrated

in literature that these chemicals can increase the cold viscosity of a starch paste (Azizi, et al., 2005; Moorthy, 1985). The necessity of using this surfactant should be evaluated. These chemicals are added to prevent foam formation, but *L. amylophilus* do not need any oxygen feeding and do not produce any gas, so this culture should not tend to produce foam.

The fermentation of sterilized media, without pre-heating, performed with an agitation of 100 RPM for potato waste starch and 50 RPM for commercial potato starch were the optimal configurations for this study. The results obtained can be hardly compared with existing literature. In fact, there are no publications that used *L. amylophilus* NRRL B-4437 to ferment potato starch. Only four publications report the use of this strain: Pierre Mercier (1990), P. Mercier et al. (1992), Nakamura (1979) and Pompeyo et al. (1993). Also, lactic acid production rate depends on growth and enzyme affinity with the substrate, which depend on the starch type but also on the medium preparation method. From our experiments, it is not possible to determine an absolute yield, since the real initial starch concentration is not known. It is however possible to conclude, from the results obtained, that potato waste starch can have a greater potential for lactic acid fermentation than commercial potato starch. The feasibility of producing lactic acid by fermenting potato waste starch with *L. amylophilus* has been demonstrated on a microbiological basis, as lactic acid concentration reached are close to the one obtained on an optimal medium, the glucose one. The first objective has been completed.

2.2 Process parameters and flowsheets

The next section presents the fermentation parameters and flowsheets of industrial lactic acid production from potato wastewater and from corn, which use as a fermentation substrate potato waste starch and dextrose respectively. In the case of lactic acid produced by the direct fermentation of potato waste starch, these are expected parameters, since there is no existing industrial facility. In the case of lactic acid fermentation of dextrose from corn, these are the parameters assumed for a conventional facility. Even if industrial-scale facilities which ferment dextrose from corn do exist, this information cannot be obtained from lactic acid producers due to

confidentiality reasons. Therefore, these parameters are assumptions, based on literature and general knowledge in bioengineering from scientists in this field. The fermentation parameters detailed description and assumptions/references related to it are presented in Appendix D.

2.2.1 Lactic acid from potato waste starch

Table 2-1 presents the assumed parameters for the fermentation process:

Table 2-1: Parameters for the fermentation of potato waste starch from wastewater

Fermentation parameter	***Value/set***
Mode	Batch
Concentration of substrate	40 g dry PWS/L
Time length	3 days
Final lactic acid yield	0.9 g lactic acid/g dry PWS
pH	6
Temperature	37°C
Nitrogen nutrient source composition	45 g CSL solids/L
Pretreatment of media	Gelatinization at 90°C
Agitator	Rushton turbine(s)
Power of agitation	Scale-up: P/V constant from laboratory experiments

The major steps in the process of lactic acid production from potato waste starch are presented below:

1. Concentration of starch in potato wastewater to 13% of humidity;
2. Fermentation of potato waste starch media with *L. amylophilus*;
3. Lactic acid purification.

The process flowsheet for lactic acid fermentation and its purification (step 2 and 3) is presented in Figure 2-10. Pumps, utilities and residence tanks are not shown.

Concentration of waste starch in potato wastewater to 13% of humidity

A detailed process flowsheet for waste starch concentration is presented in Figure 2-9. This process was the one employed for the recuperation of the starch residue used in the present study experiments.

First, starch wastewater and grinded rejected potatoes pass through a vibrating screen for the recuperation of bigger solid residues. Those residues may be pieces of peel or potato flesh and are given to farmers for animal alimentation. The starch solution ends up in a residence tank (see "Tank 1" in Figure 2-9) after which it is centrifuged through hydrocyclones. The solid phase, the starch residue, is conducted to a vacuum drum, while the liquid is redirected to another residence tank (see "Tank 2" in Figure 2-9). To optimize starch recovery efficiency, this liquid is centrifuged a second time. The centrifuged liquid will end up in a drain, to be treated by the wastewater treatment facility. A vacuum drum will lower the water content of starch residue around 40%. To reach a lower moisture content, starch is transferred to an air lift dryer, from which the moisture content is reduced to at least 13%, the aimed set value. This level of humidity enables a sufficient conservation of the product.

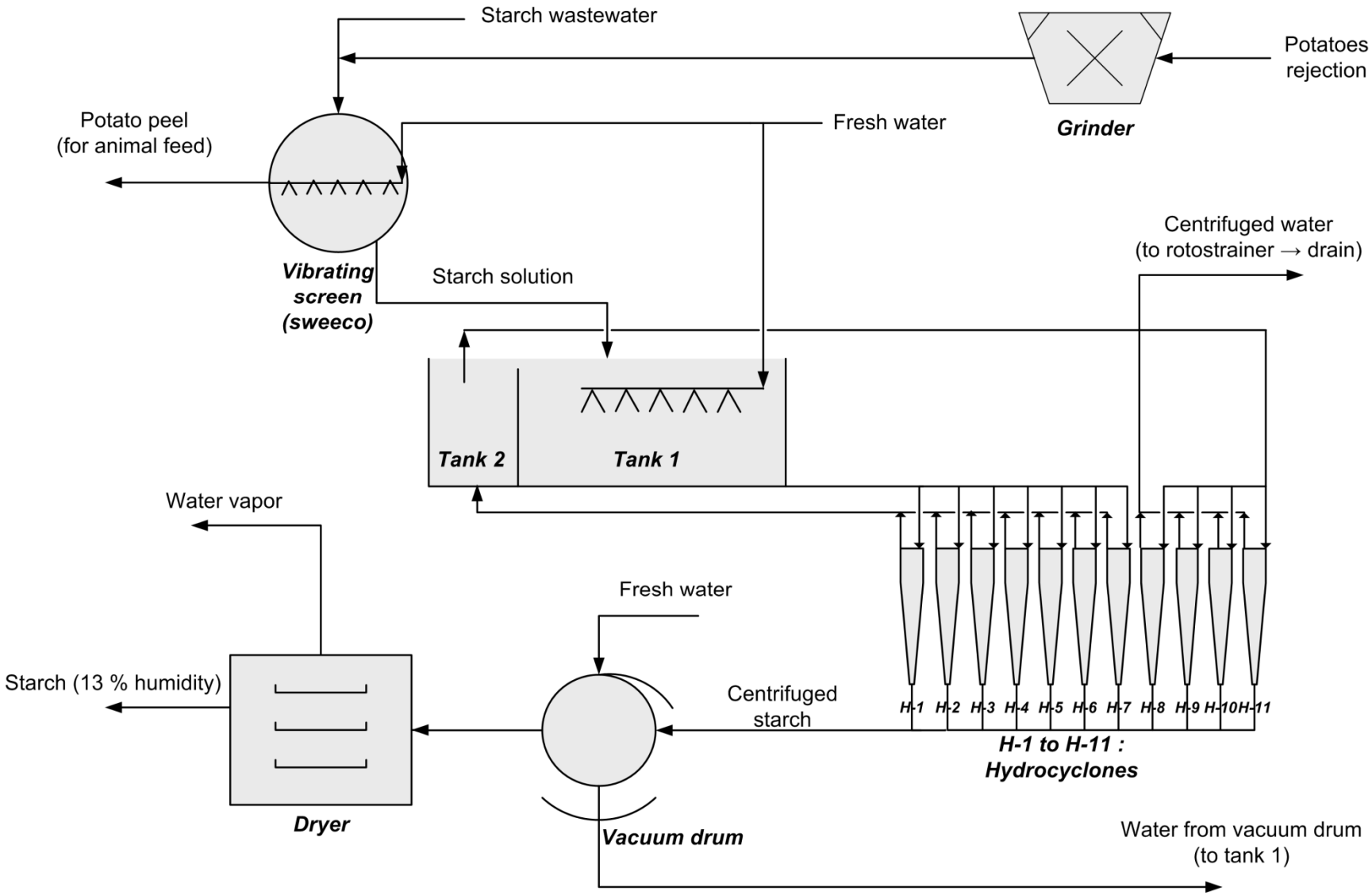

Figure 2-9: Potato starch waste concentration flowsheet

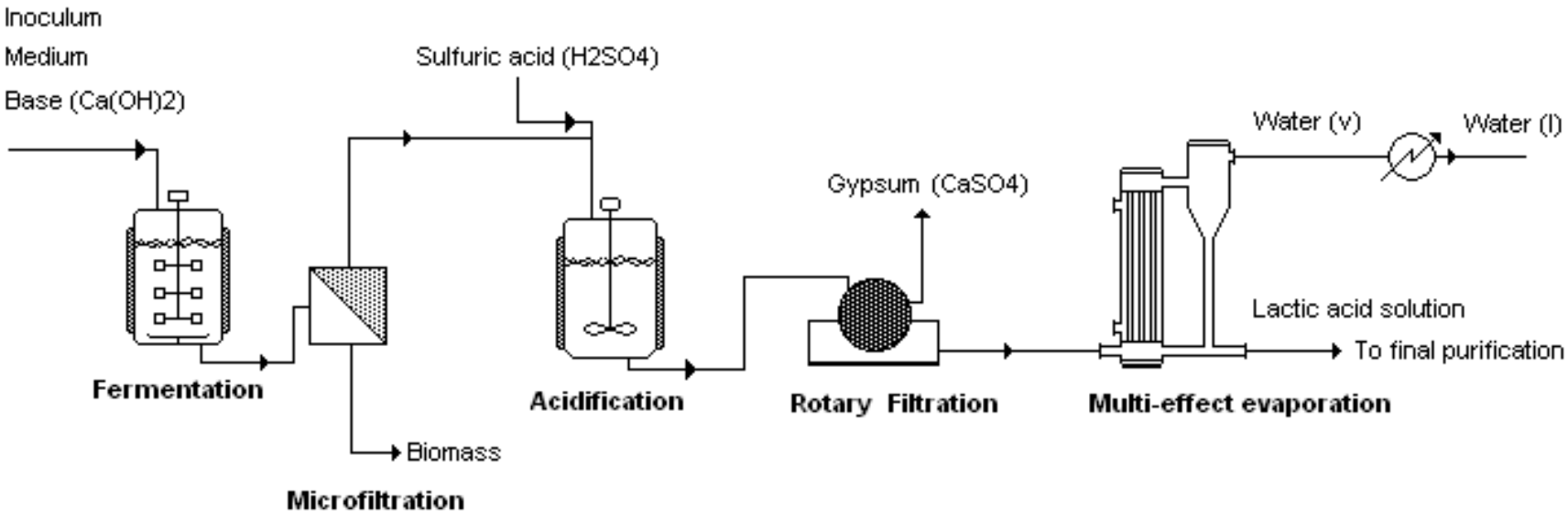

Figure 2-10: Process flowsheet for lactic acid production and purification

Modified from SuperPro Designer® (Intelligen Inc., 1991)

The fermentation of starch media

The medium (water, starch residue and nutrients) is initially fed to the fermentor. As mentioned before, due to its compact form and its insolubility at ambient temperature, starch has to be gelatinized to improve its hydrolysis rate. This step is realized directly in the fermentor, with a proper agitation to maintain starch dispersion. Steam and water are respectively fed to the jacket/coil of the fermentor for heating to 90ºC, and then to cool the media to 37ºC, the set fermentation temperature.

Fermentations are realized in a batch mode and are inoculated with a preculture of *L. amylophilus* (inoculum). The culture medium is maintained at 37°C with the help of water through the jacket/coils of the fermentor. The pH is maintained at 6 with calcium hydroxide. Starch is saccharified into dextrose, and dextrose is assimilated by bacteria to produce biomass and lactic acid. At pH 6, lactic acid is essentially all under its dissociated form (Carlson, et al., 2002). For the sake of simplification, it will be considered that 100% of lactic acid was under the calcium lactate form. The reactions occurring are presented below:

- *Saccharification of starch into glucose molecules:*

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6$$

- *Fermentation of glucose into lactic acid:*

$$C_6H_{12}O_6 \xrightarrow{biomass} 2C_3H_6O_3 + biomass$$

- *Calcium lactate formation:*

$$2C_3H_6O_3 + Ca(OH)_2 \rightarrow C_3H_5O_3^-Ca^+O_3^-H_5C_3 + 2H_2O$$

Since gelatinization and fermentation are realized in the same unit, only one fermentor is shown on the flowsheet.

The purification of lactic acid

The purification steps are based on Vink et al. (2007). Lactic acid is purified by the dissociation of calcium lactate with sulfuric acid, which leads to the formation of gypsum (calcium sulphate; $CaSO_4$), following the reaction shown in the equation below:

$$C_3H_5O_3^-Ca^+O_3^-H_5C_3 + H_2SO_4 \rightarrow CaSO_4 + 2C_3H_6O_3 \quad \text{(Datta, 2007)}$$

Even if some new methods are being developed to reduce the production of waste like gypsum, this purification method is presented in NatureWorks and Purac last published flowsheet (Purac, 2009; Vink, et al., 2007). For this reason, the basic purification process with sulfuric acid, which might has been improved in the industry by solvent addition (Joglekar, et al., 2006), was chosen. The four major steps of the purification process are:

- Removal of biomass through microfiltration;
- Precipitation of gypsum to dissociate lactic acid from calcium, with the help of sulfuric acid;
- Removal of gypsum with a rotary filter;
- Removal of water by a multi-effect evaporator, to lead to a 88 wt% lactic acid solution, neglecting final impurities. The final concentration of lactic acid is based on USP/FCC grade (United States Pharmacopeial/Food Chemical Codex) (González, et al., 2007).

The purification steps follow well-known heuristics (“rules of thumb”) in bioseparation processes, taken from Bioseparations Science and Engineering (2003), chapter 11:

- Remove the most plentiful impurities first;

- Remove the easiest-to-remove impurities first;
- Make the most difficult and expensive separations last;
- Select processes that make use of the greatest differences in the properties of the product and its impurities.

In accordance with the first two heuristics presented and to limit fouling of units, it has been decided that biomass would be removed by microfiltration, prior to acidification. The microfiltration is preferred to centrifugation in the industry, due to its low operational cost (Arcand, 2010). Following the acidification step, gypsum is removed by rotary filtration, as suggested by NatureWorks (Vink, et al., 2007). Other insoluble parts may be removed by the filtration units. Concentration of lactic acid follows. Since high quantity of water has to be removed, it is expected that multi-effect evaporators are used in the industry (Bohlmann, 2004). The evaporator will be assumed as having 3 effects. Final purification, to remove trace of water and soluble contaminants, such as proteins and sugars, follows.

2.2.2 Lactic acid from corn

Table 2-2 presents the assumed parameters for the fermentation step:

Table 2-2: Parameters for fermentation of dextrose from corn

Fermentation parameter	***Value/set***
Mode	Batch
Concentration of substrate	92 g dextrose/L
Time length	3 days
Final lactic acid yield	0.9 g lactic acid/g dextrose
pH	6
Temperature	45°C
Nitrogen nutrient source composition	45 g CSL solids/L
Pretreatment of media	None
Agitator	Rushton turbine(s)
Power of agitation	Scale-up: P/V constant from laboratory experiments

The major steps in the process of lactic acid production from corn are presented below:

1. Production of glucose from corn;
2. Fermentation of glucose media with *L. amylophilus*;
3. Lactic acid purification.

The general process flowsheet related to the lactic acid fermentation and purification (step 2 and 3) is the same as the one presented in Figure 2-10.

Production of dextrose from corn

Once corn has been harvested, starch can be extracted and transformed into sweeteners like dextrose with the help of an acid or enzymatic hydrolysis. This process, the “corn wet milling”, leads to different products from starch, but produces inevitably co-products, which are sold as animal additives, as mentioned in the state-of-art (section 1.3). A typical process flowsheet of a corn wet milling facility can be found in Blanchard (1992).

The fermentation of glucose media

The medium (water, residue starch and nutrients) are initially fed to the fermentor. No pretreatment, even a heat treatment, is given to the media. The fermentor is then inoculated with a lactic acid bacteria preculture, which is not amylolytic. The medium is maintained during fermentation at 45°C with water through the jacket/coils and at a pH of 6 with calcium hydroxide. As mentioned previously, calcium lactate is formed during the neutralization of lactic acid.

The purification of lactic acid

The same method of purification as the one described in section 2.2.1 has been chosen.

CHAPTER 3 LIFE CYCLE ASSESSMENT

3.1 Goal of the study

This life cycle assessment (LCA) study investigates, from an environmental point of view, the development of a new process for poly-$_L$-lactic acid (PLLA) production. Its goal is to compare the potential environmental impacts of producing PLLA via the direct valorization of an agribusiness waste (potato waste starch) with the utilization of transformed foodstuff (dextrose from corn). Particularly, this evaluation will be carried out through a comparative "cradle-to-gate" LCA of two different processing scenarios for lactic acid production. These scenarios are based on the processes presented in chapter 2. This study will demonstrate, through an impact assessment, the potential environmental benefits/disadvantages that could be reached in the near-future by producing lactic acid for PLLA synthesis by fermenting directly concentrated potato waste starch with *Lactobacillus amylophilus* instead of dextrose from a corn wet milling facility. It will also present some limitations related to this innovative process and subsequent potential optimizations that could be done to improve the ecobalance of PLLA produced through this process configuration.

This LCA is addressed to industrial developers, which want to improve the ecobalance of PLLA processing. All emissions and energy consumption data related to potato waste starch concentration were provided by an industrial partner, who gave a residue sample for the previous laboratory experiments. Due to a confidentiality agreement, the partner's name cannot be disclosed.

3.2 Scope of the study

The framework of this study is set according to the ISO 14040 and 14044 guidelines (ISO, 2006a, 2006b). The function of the product systems is defined as producing lactic acid for synthesis of a polymer used in food packaging. **The functional unit is to produce 1 kg of $_L$-lactic acid solution, at 88% wt** (USP/FCC grade concentration (González, et al., 2007))**.** The scenario boundaries are shown in Figure 3-1 and 3-2.

This “cradle-to-gate” LCA includes all steps, from the production and extraction of raw materials and fuels, followed by all conversion and processing steps to produce $_{L}$-lactic acid for the synthesis of PLLA (lactic acid fermentation and purification), until this product is delivered out of the lactic acid factory gate. In this work, the scenario called “Corn” corresponds to the process using corn as a raw material; the scenario “PWS water”, using potato wastewater. Each scenario has four major steps: media preparation, media pre-heating, lactic acid fermentation and lactic acid purification. The media preparation consists of the substrate preparation for fermentation. In the “Corn” scenario, this is the production of dextrose from corn through the corn wet milling process, including the agricultural steps for corn growing. In the “PWS water” scenario, this includes the concentration of potato waste starch out of potato wastewater. All inputs related to the media constitution are also included in the media preparation step, such as corn steep liquor and water. Part of the media is used for cellular growth (preparation of the inoculum). Media pre-heating corresponds to any heating of the media prior to fermentation: heating to fermentation temperature in the case of the scenario “Corn”; gelatinization and cooling to fermentation temperature in the case of the scenario “PWS water”. The lactic acid fermentation and its purification follow. The purification step includes the biomass filtration, the calcium lactate dissociation with sulphuric acid, the filtration of gypsum and finally the concentration of lactic acid to 88% wt.

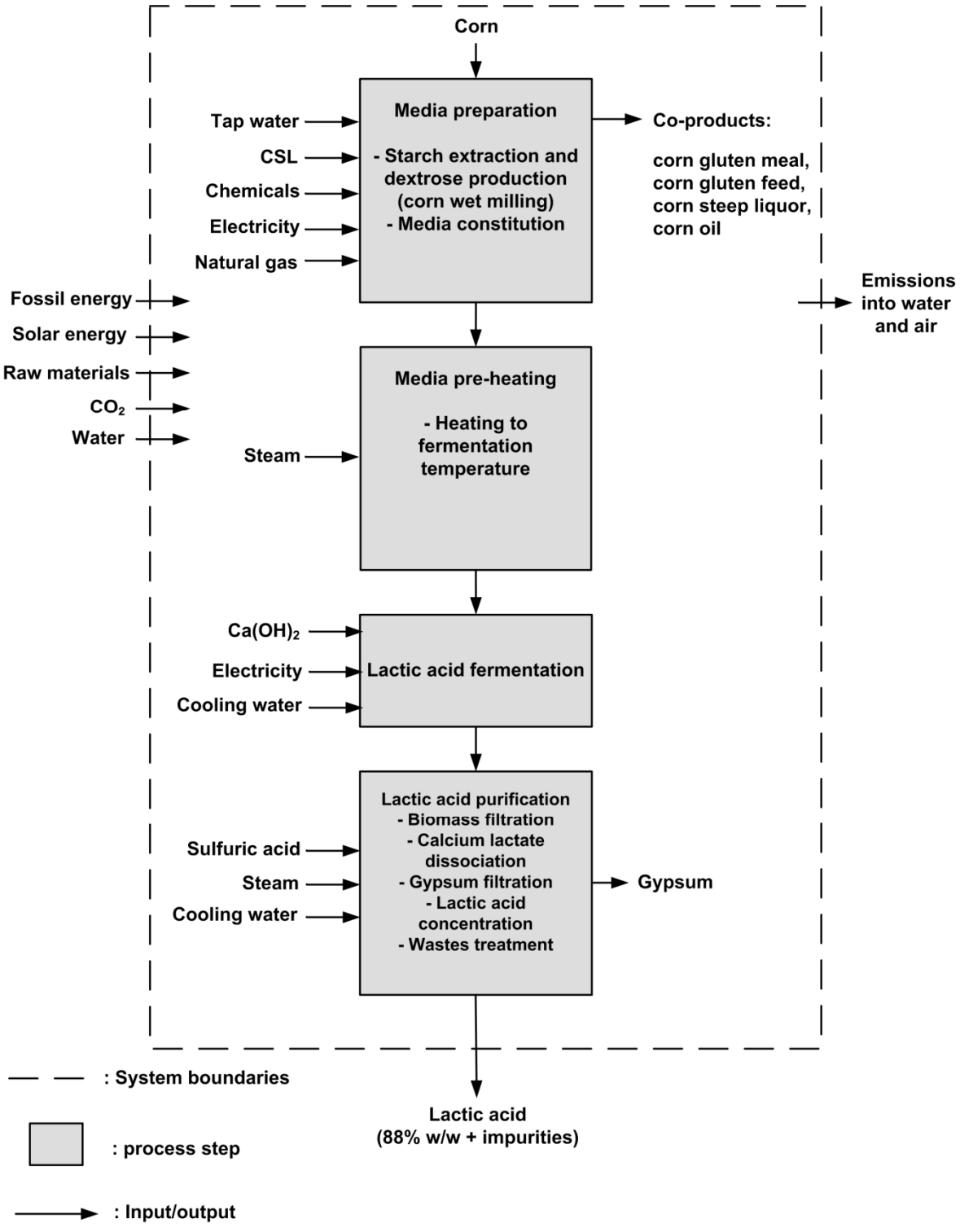

Figure 3-1: System boundaries of the “Corn” scenario

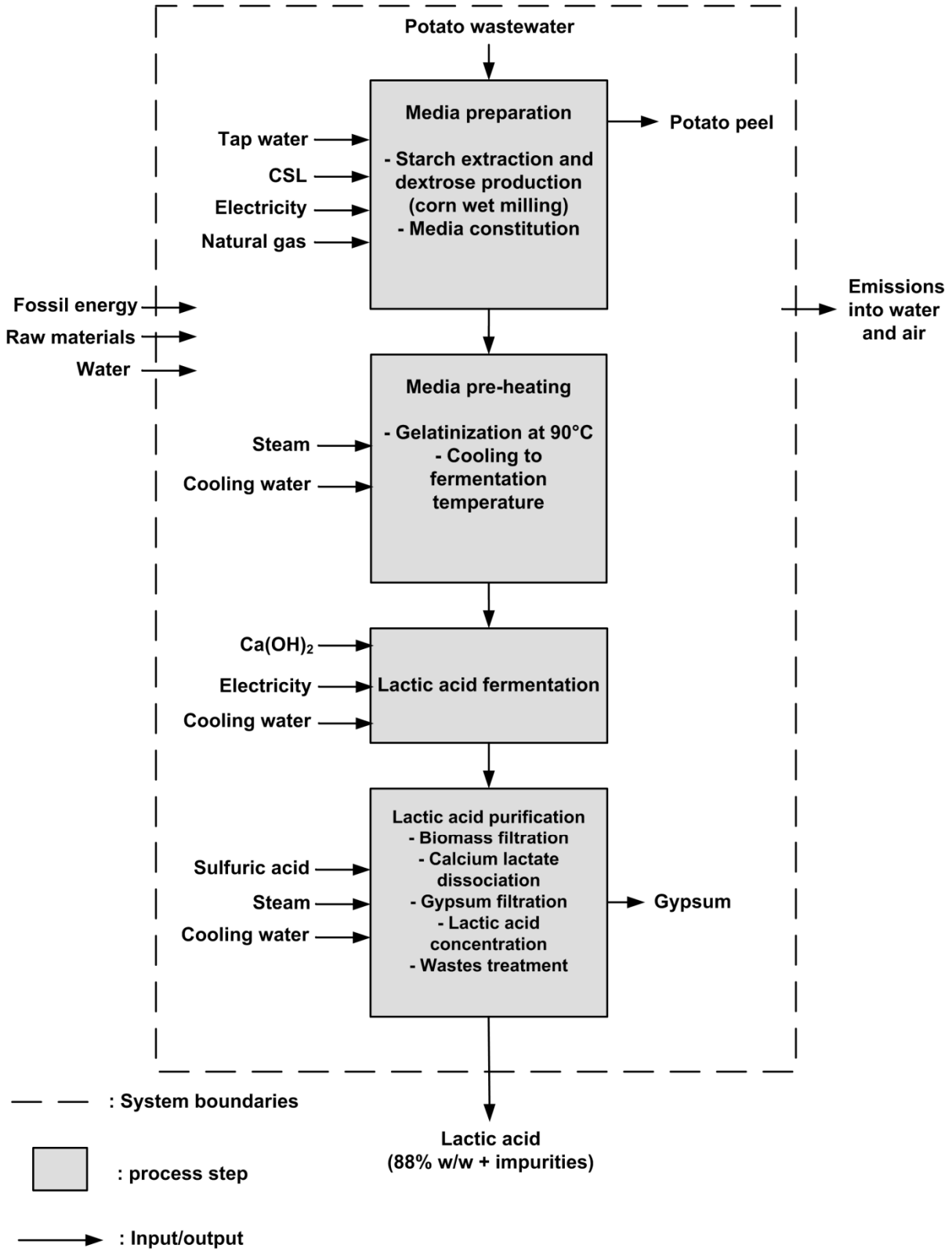

Figure 3-2: System boundaries of the “PWS water” scenario

The transport of the raw materials and sludge to the landfill is not detailed in Figures 3-1 and 3-2, but is, however, included. The wastewater treatment and the final disposal of biomass are also included. Excluded processes are presented below:

1. This study does not further compare the polymerization steps following the lactic acid purification because they are assumed to be exactly the same for both processes once lactic acid has been purified. Any modification to the actual polymerization process for PLLA is not part of this study;
2. To obtain a lactic acid of high purity (water-white), it is expected that lactic acid will be esterified with methanol or ethanol, distillated and hydrolyzed with water, like the conventional process (Datta, et al., 2006). The associated environmental impacts of the esterification/hydrolysis step are expected to be identical for both product systems;
3. Removal of soluble impurities can be performed by ultrafiltration or by carbon treatement. The contribution of these filtration units to the environmental impacts is expected to be very low (Bohlmann, 2010) and so is not supposed to be environmentally relevant;
4. Cooling of the different vapor condensates and heat exchanger waters;
5. Water consumption related to cleaning and washing of the different units can be hardly evaluated without pilot-plant experiments;
6. The electrical consumption related to utilities and pumps in the lactic acid fermentation/purification process, except agitation during fermentation, for lack of a detailed process design;
7. Cell bank preparation and energy consumption related to inoculum propagation, as it is the case in some economical assessments (Akerberg, et al., 2000);
8. The lactic acid process infrastructure is supposed to be negligible;
9. Minerals and other chemicals added to the media, since their concentration over the nitrogen source (corn steep liquor) is expected to be low (< 10% wt of total nutrients (Carlson, et al., 2002)) and none of them contain heavy metals.

The allocation methods for potato waste starch, potato peel, dextrose and corn steep liquor are described below:

- ✓ Potato wastewater et potato peel (economic allocation)

Potato waste starch is recuperated from wastewater with the help of different concentration steps. Since potato wastewater has no economical value, none of the emissions and primary energy of the potato chip process is allocated to this waste, even for the treatment of its water. Concentration of potato wastewater puts an economical value to potato waste starch, by removing water and these steps are not necessary for the chip production, the main product of the industrial partner. In this case, emissions and energy consumption related to potato waste starch concentration have to be allocated to potato waste starch. All inputs/outputs related to this part (natural gas, electricity and tap water for washing and its treatment) of the potato chips process were given by the industrial partner. Reference flows per kg of potato waste starch were calculated from it.

Potato peel filtrated from wastewater are given to farmers in the immediate vicinity of the plant. Since it has no economical value, it is considered as a by-product. No emissions/energy of the potato waste starch concentration process will be allocated to these solid residues. Transport of the potato peel is provided by the farmers and so does not have to be considered in the product systems.

- ✓ Corn gluten meal, corn gluten feed, corn oil and corn steep liquor (system expansion)

Since different co-products, which have all an economical value, are produced in a corn wet milling facility, part of the emissions and energy consumption related to dextrose production, including corn growing and harvesting, has to be allocated to each of these co-products. The allocation will be avoided by realizing a system expansion. The co-products will be considered as replacing the production of other products, such as animal feed and oil, i.e. barley, soybean meal and soybean oil. The energy and emissions saved by this product displacement will be attributed to dextrose production.

Since the corn steep liquor used for fermentation is also a co-product of the corn wet milling process, the associated environmental impact will be from the product it normally displaces. Displaced products amounts are presented in Figure 3-3 and are based on protein content (see Appendix G for calculations). A sensitivity analysis in section 3.5.2 will compare the system expansion method with the economical allocation factors for all co-products.

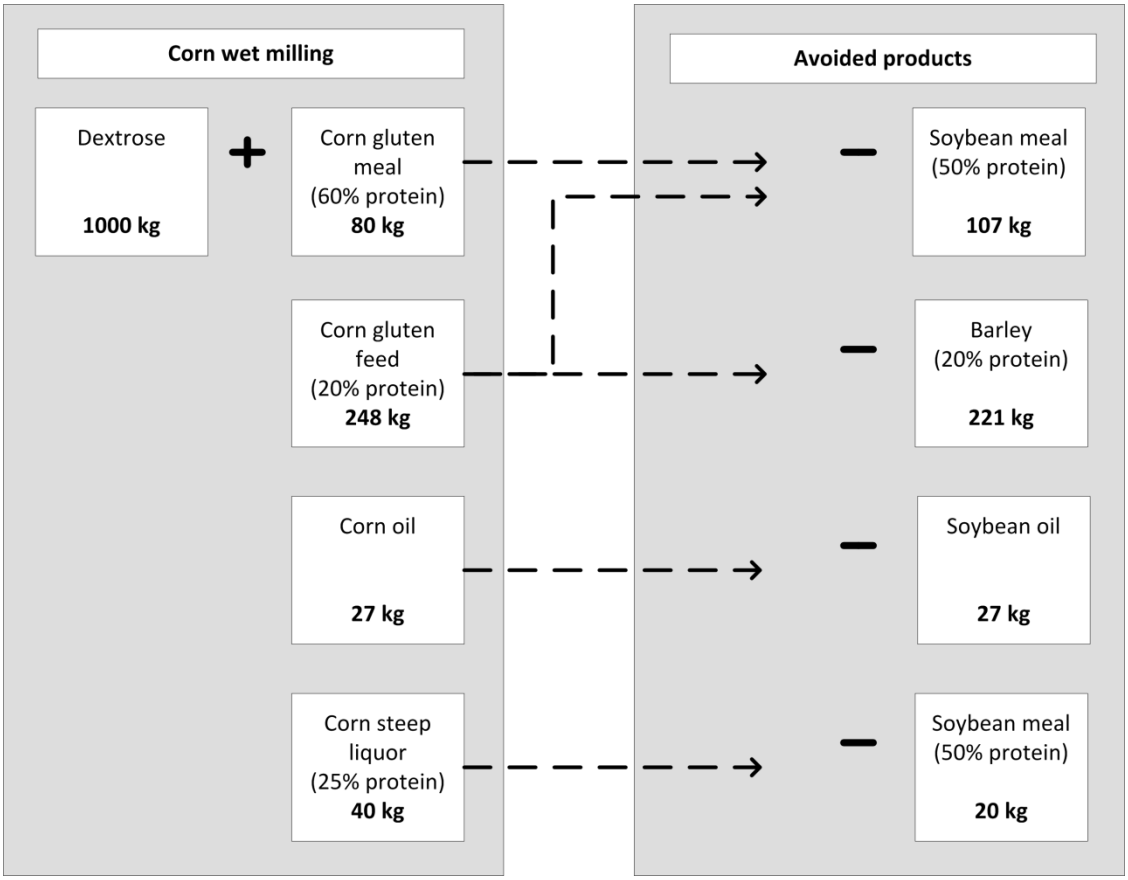

Figure 3-3: Avoided burdens for dextrose production through system expansion

3.3 Reference flows

The reference flows inventory of $_{L}$-lactic acid produced from corn or potato wastewater is based on the mass and energy flows of the process flowsheets presented in chapter 2. The mass and energy balances and the assumptions related are presented in Appendix E.

The reference flows inventory for both scenarios studied are presented in Tables 3-1 and 3-2. The assumptions related to these inventories are:

1. Water from saturated steam condensate is considered as clean since it does not come in contact with the media and so it does not need any treatment;
2. Even if it was assumed that no lactic acid ends up in the vapor phases of the multi-effect evaporator, it will be considered that the condensate from the concentrated solution has to be treated in a wastewater facility (worst case scenario philosophy);
3. Filtrated biomass (wet weight) and the residuals it contains end up in a sanitary landfill. This waste is called “sludge”;
4. Gypsum (pure, dry weight) co-product is used for land-application, as assumed in NatureWorks LCA (Vink, et al., 2007). It replaces mined gypsum. Humidity and impurities contained are not removed;
5. Water emission from the potato waste dryer are not considered as containing any pollutants;
6. To be representative of the European geography-scale, 300 km of transport will be assumed for any inputs of raw materials and 100 km of transport for sludge to the sanitary landfill. Since the corn wet milling facilities are expected to be situated relatively close to crop fields, 100 km will also be assumed for corn transportation.

Table 3-1: Inputs and outputs for the synthesis of lactic acid via the "Corn" scenario, per kg of lactic acid at 88% wt produced (neglecting final impurities)

Step	***Input***	***Value***	***Output***	***Value***
Media preparation	Tap water (15°C)	10 kg	Barley	0.22
	Dextrose$^{100\%*}$	1.0 kg	Soybean meal	0.13
	CSL	0.97 kg	Soybean oil	0.027
	Transport	0.59 tkm		
Media pre-heating	Saturated steam (121°C, 1.1 bar)	0.66 kg		
Fermentation	Calcium hydroxide	0.37 kg		
	Cooling water	1.7 kg		
	Electricity	0.0015 kWh		
	Transport	0.11 tkm		
Purification	Sulphuric acid	0.49 kg	Gypsum (pure, dry weight)	0.67 kg
	Saturated steam	4.2 kg	Sludge	0.12 kg
	Cooling water	390 kg	Wastewater	11 kg
	Transport	0.16 tkm	Lactic acid (88% wt) + impurities	1.0 kg

*: Inputs/outputs are available per kg of dextrose produced from corn crop at a corn wet milling facility. "Dextrose$^{100\%}$" represents the inputs and outputs per kg of dextrose produced, with 100% allocation to it.

Table 3-2: Inputs and outputs for the synthesis of lactic acid via the "PWS water" scenario, per kg of lactic acid at 88% wt produced (neglecting final impurities)

Step	***Input***	***Value***	***Output***	***Value***
Media preparation	Tap water (15°C)	24 kg	Potato peel	Unknown
	PWS (humid)*	1.1 kg		
	CSL	2.2 kg		
	Transport	1.0 tkm		
Media pre-heating	Saturated steam (121°C, 1.1 bar)	3.7 kg		
	Cooling water	270 kg		
Fermentation	Calcium hydroxide	0.37 kg		
	Cooling water	3.8 kg		
	Electricity	0.011 kWh		
	Transport	0.11 tkm		
Purification	Sulphuric acid	0.48 kg	Gypsum (pure, dry weight)	0.67 kg
	Saturated steam	10 kg	Sludge	1.0 kg
	Cooling water	870 kg	Wastewater	24 kg
	Transport	0.25 tkm	Lactic acid (88% wt) + impurities	1.0 kg

*: Inputs/outputs are available per of kg potato waste starch (13% humidity) produced from the concentration of potato wastewater

3.4 Life Cycle Inventory analysis

3.4.1 Development of new process datasets

The Ecoinvent life cycle inventory database (Frischknecht, et al., 2004) was chosen for the conversion of reference flows in term of cradle-to-gate emissions and primary energy inventory. For the substrate and nutrients used in fermentation, which are dextrose from corn, potato waste starch and corn steep liquor, there are no existing processes in Ecoinvent. Specific processes were created per kg of substrate/corn steep liquor. These processes are based on literature and industrial data. Processes created for the purpose of this study are presented below. Table 3-3 presents the inputs and outputs related to dextrose production from corn, with 100% allocation to dextrose, taken from Renouf et al. (2008) LCA study.

Table 3-3: Inputs and outputs for the processing of corn per ton of dextrose (100% allocation to dextrose)

Input	***Unit***	***Value***	***Output***	***Unit***	***Value***
Corn	t	1.5	$Dextrose^{100\%}$	t	1.0
Electricity	MJ	322	Air emissions		
Natural gas	MJ	1678	Particulate (PM10)	g	0.7
Lime	kg	0.3	Water emissions		
Sulphuric acid	kg	0.45	BOD_5	g	0.2
Sulphur dioxide	kg	3.06	Chlorides	g	118.8
Urea	g	208	Sulphate	g	0.2
Sodium hydroxide (50%)	g	282	Suspended matter	g	0.7
Sodium chloride	g	65			
Cyclohexane	g	55			
Chlorine	g	12			
Water	t	4.9			
Transport	tkm	1.5E+02			

Table 3-4 presents the input and output of corn steep liquor production, taking into account the system expansion methodology.

Table 3-4: Input and output for the processing of corn per ton of corn steep liquor

Inputs	***Unit***	***Value***	***Output***	***Unit***	***Value***
Soybean meal	t	0.5	Corn steep liquor	t	1.0

The inputs/outputs related to the potato waste starch production from wastewater concentration are given in Table 3-5. These are based on annual averages of the water and energy consumption for potato waste concentration by the industrial partner in 2009.

Table 3-5: Inputs and outputs for the concentration of potato wastewater per ton of potato waste starch (13% humidity)

Inputs	***Unit***	***Value***	***Output***	***Unit***	***Value***
Potato wastewater (1)	t	Unknown	Potato waste starch (humid)	t	1.0
Electricity	kWh	3.4E+02	Wastewater from (1)	t	Unknown
Tap Water (2)	t	16	Wastewater from (2)	t	16
Natural gas	GJ	3.9			

3.4.2 Main data sources

As previously mentioned, the Ecoinvent database (Frischknecht, et al., 2004) was chosen. It has been assumed that both product systems are situated in Europe. All the processes of the systems represent the European context, except for corn, soybean meal, soybean oil and barley, which used US data. Since wastewater and solids treatment are included in the product systems, the processes related to these outputs are their treatments. Table 3-6 presents the Ecoinvent processes associated with the reference flows.

Table 3-6: The corresponding Ecoinvent processes for the reference flows

Reference flow	*Unit*	*Ecoinvent process*
Barley	kg	Barley grains IP, at farm/CH U
Calcium hydroxide	kg	Lime, hydrated, packed, at plant/CH U
Chlorine	kg	Chlorine, liquid, production mix, at plant/RER U
Corn	kg	Corn, at farm/US U
Cyclohexane	kg	Cyclohexane, at plant/RER U
Electricity	kWh	Electricity, medium voltage, production RER, at grid/RER U
Gypsum	kg	Gypsum, mineral, at mine/CH U
Lime	kg	Limestone, milled, packed, at plant/CH U
Natural gas	MJ	Natural gas, burned in industrial furnace >100kW/RER U
Saturated steam	kg	Steam, for chemical processes, at plant/kg/RER
Sludge (landfill)	kg	Process-specific burdens, sanitary landfill/CH U
Sodium chloride	kg	Sodium chloride, powder, at plant/RER U
Sodium hydroxide (50%)	kg	Sodium hydroxide, 50% in H2O, production mix, at plant/RER U
Soybean meal	kg	Soybean meal, at oil mill/US U
Soybean oil	kg	Soybean oil, at oil mill/US U
Sulphur dioxide	kg	Sulphur dioxide, liquid, at plant/RER U
Sulphuric acid	kg	Sulphuric acid, liquid, at plant/RER U
Transport	t·km	Transport, lorry >32t, EURO3/tkm/RER
Urea	kg	Urea, as N, at regional storehouse/RER U
Tap water/cooling water	kg	Tap water, at user/RER U
Wastewater (treatment)	m^3	Treatment, sewage, unpolluted, to wastewater treatment, class 3/m3/CH

As previously mentioned, potato wastewater, the treatment of its water and potato peel do not have any energy/emissions associated with them so no inventory datasets are needed for these inputs/output.

3.5 Impact assessment

The IMPACT 2002+ (v. 2.05; Jolliet et al. (2003)) method was used to simulate the potential environmental impacts of both scenarios. Simulations were performed with the help of SimaPro 7.1. The impact simulation results are based on a multi-criteria approach of damage characterization, with a focus on resources depletion (primary energy consumption). The other impact categories are human health, ecosystem quality and climate change.

3.5.1 Results

Figure 3-4 to 3-7 present the potential impacts of the "Corn" and "PWS water" scenarios for the resource depletion, climate change, ecosystem quality and human health impact categories, in each of the main processing steps.

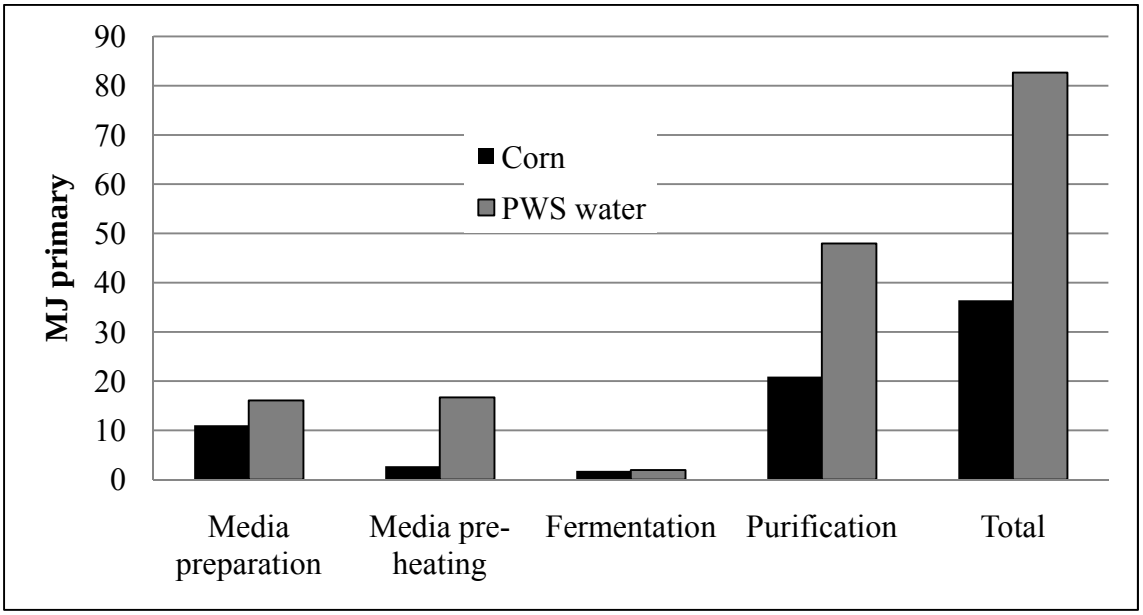

Figure 3-4: Impacts on resource depletion, per kg of lactic acid

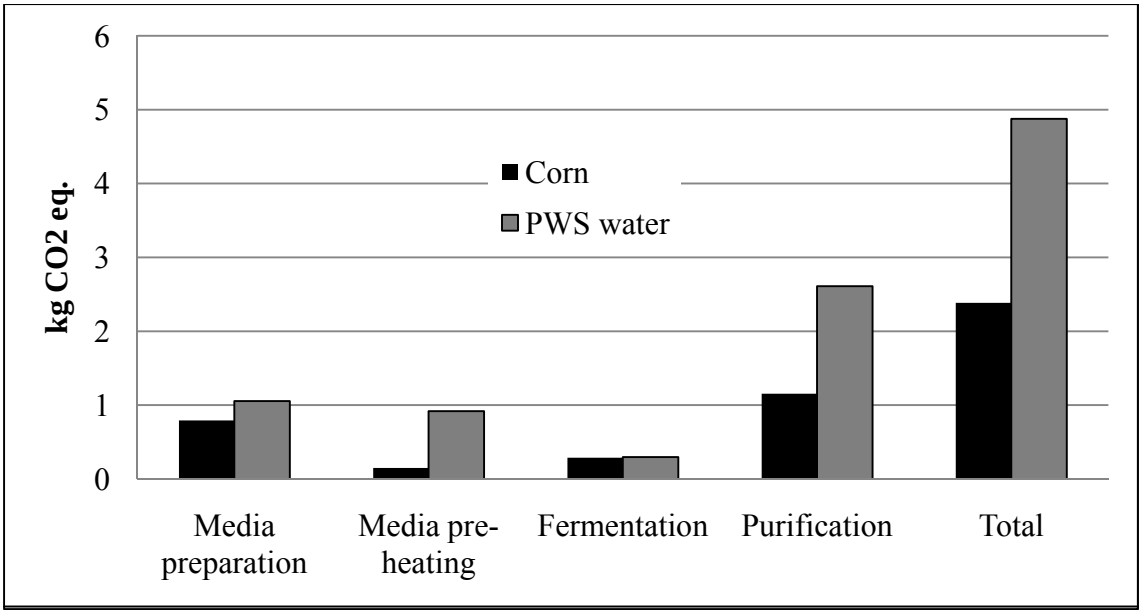

Figure 3-5: Impacts on climate change, per kg of lactic acid

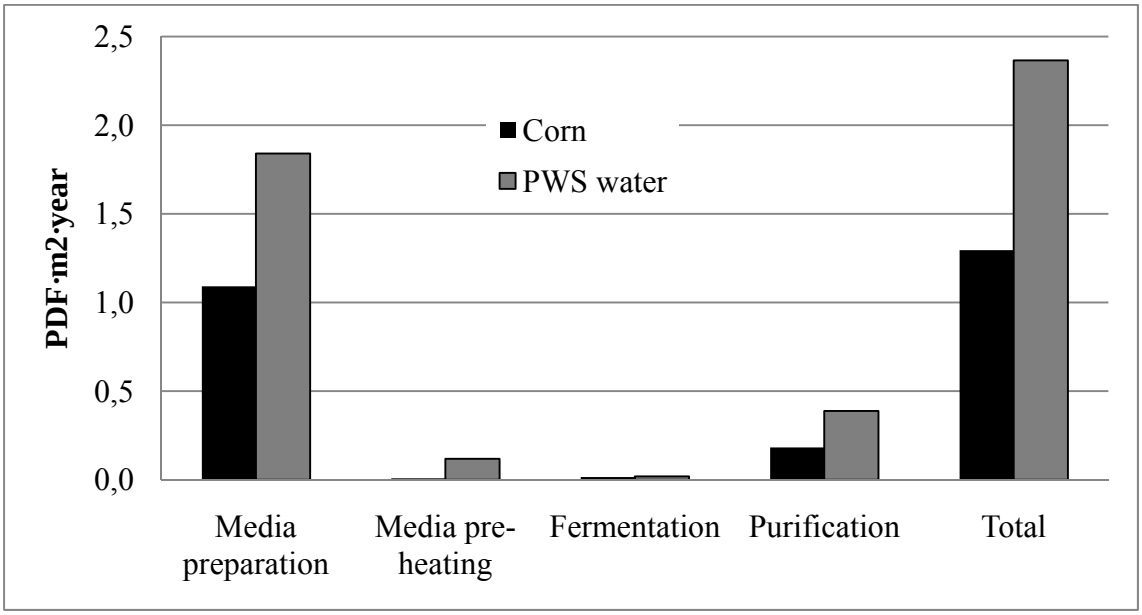

Figure 3-6: Impacts on ecosystem quality, per of kg lactic acid

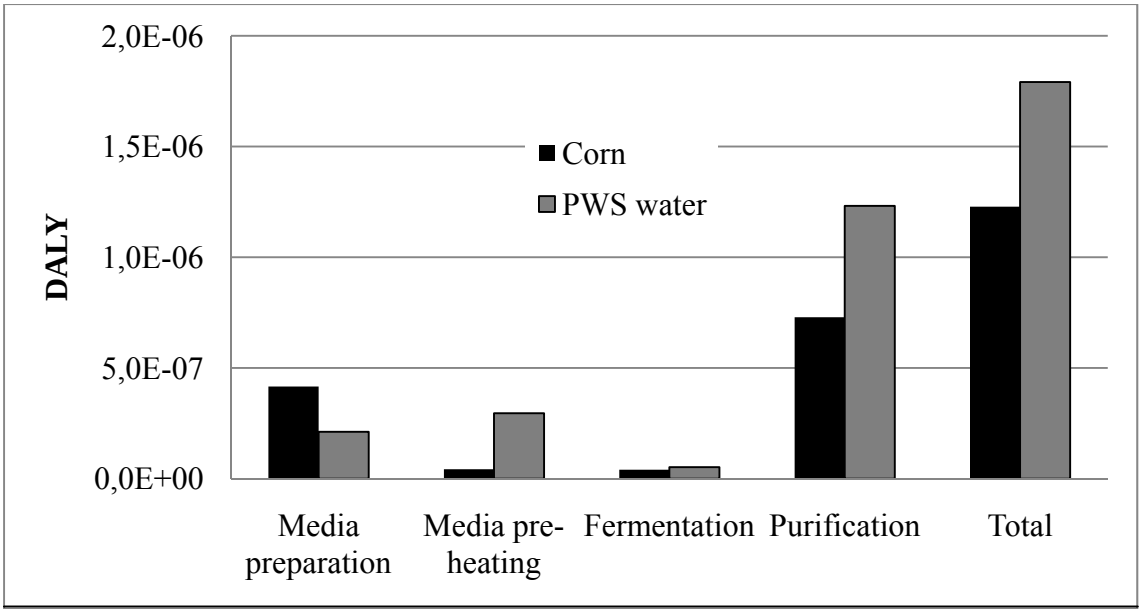

Figure 3-7: Impacts on human health, per kg of lactic acid

It can be observed from Figures 3-4 to 3-7 that the "PWS water" scenario has always a higher total impact in each damage category compared to the scenario "Corn". The purification and the media pre-heating steps are highly energy consuming, since they contribute to at least 70% more for each damage category than the corresponding steps of the "Corn" scenario. In each category, the fermentation step contribution is low and similar for both scenarios. The media preparation impacts for each end-point category are variable. For the ecosystem quality, the "PWS water" scenario has approximately 80% more impact than the "Corn" scenario; in case of resource depletion and climate change, it is more than 100%. However, in the case of the human health category, the "PWS water" scenario has half of the impact of the "Corn" scenario.

3.5.2 Interpretation

For the resource depletion impact category, the major differences between both scenarios are between the media pre-heating and the purification steps and come in a large part from the steam consumption. In fact, the "PWS water" scenario is characterized by a higher saturated steam consumption per kg of lactic acid produced for these steps. This is related to the necessity of gelatinizing starch and to the higher quantity of water to be vaporized during the lactic acid purification, which is due to the more diluted concentration of lactic acid leaving the bioreactor. Saturated steam, which is provided by heating water in a boiler operated with natural gas and heavy oil, has a direct impact on fuel consumption and, therefore, on resource depletion. In both steps, saturated steam is the predominant process for the resource depletion impact category. The higher contribution of steam over other elementary processes is even more obvious in the case of the purification step, as seen in Figure 3-8:

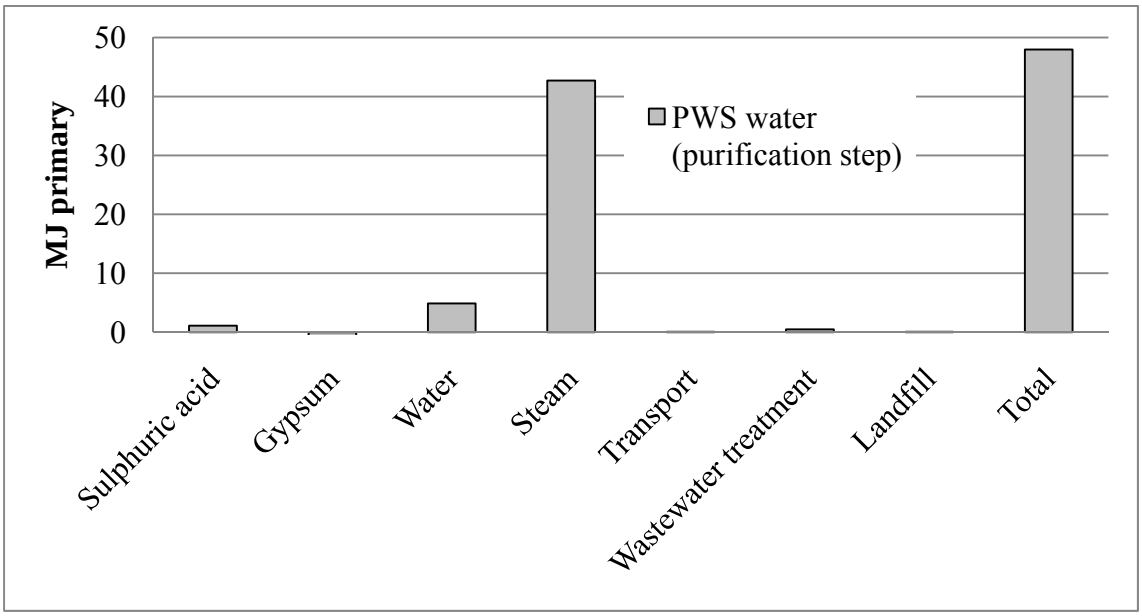

Figure 3-8: Processes contribution on the impact on resources by scenario "PWS water" during purification (/kg lactic acid)

The superiority of the purification impact over other steps in the resource impact category was expected, since purification is well-known as being the costly part of lactic acid production (Datta, et al., 2006). Energy consumption is normally a major operating cost. Reducing steam consumption in purification and media pre-heating steps, by improving the process design and configuration, should be a priority to improve the ecobalance of lactic acid produced from the direct fermentation of potato waste starch.

The primary energy consumption of the media preparation step for the "PWS water" scenario is not intuitive, since potato wastewater is considered as a waste (out of system boundaries) and its concentration is a simpler process than corn growing and wet milling. Nevertheless, the media preparation step has approximately 45% more impact on resource depletion in the case of "PWS water" scenario. The energy consumption associated with this system step is mainly due to substrate preparation (i.e. concentration of potato starch in wastewater), as observed in Figure 3-9:

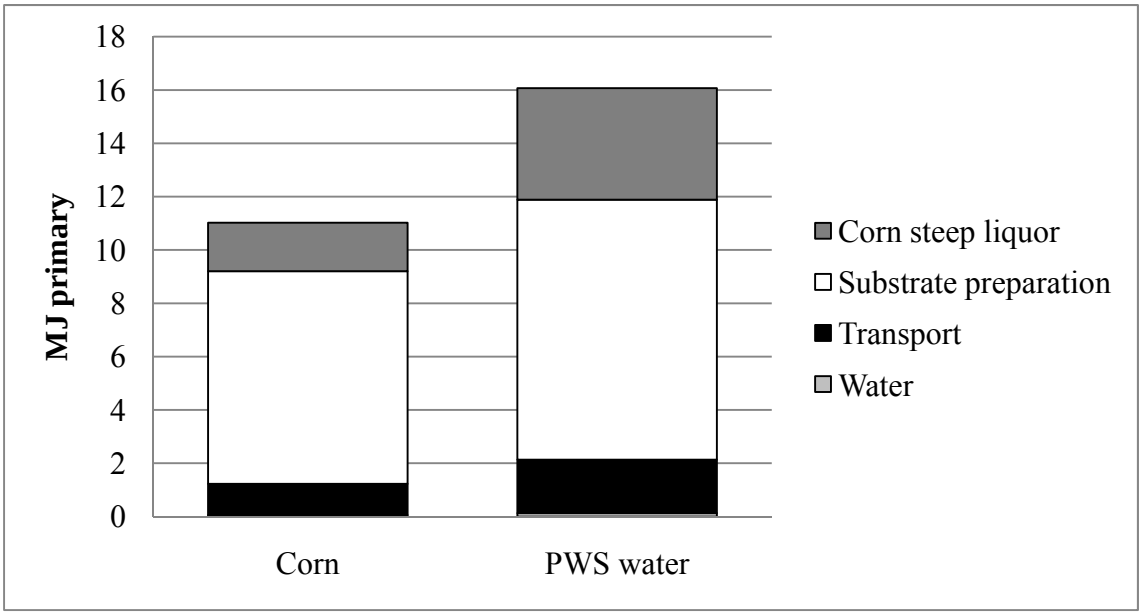

Figure 3-9: Contribution of media preparation processes in resource depletion (/kg lactic acid)

The energy consumption related to the susbtrate preparation is 8.0 MJ primary/kg of lactic acid and 9.8 MJ primary/kg of lactic acid for the "Corn" and "PWS water" scenarios respectively. Detailed results of the different elementary processes for the production of 1 kg of potato waste starch are presented in Figure 3-10. It can be observed that the final starch drying, which uses natural gas as a combustible, is the highest energy consumer (5.7 MJ primary/kg lactic acid), contributing to 58% of the resource depletion associated with the potato wastewater concentration step alone. Pumps and motors electrical energy are the next larger contributors (3.9 MJ primary/kg lactic acid).

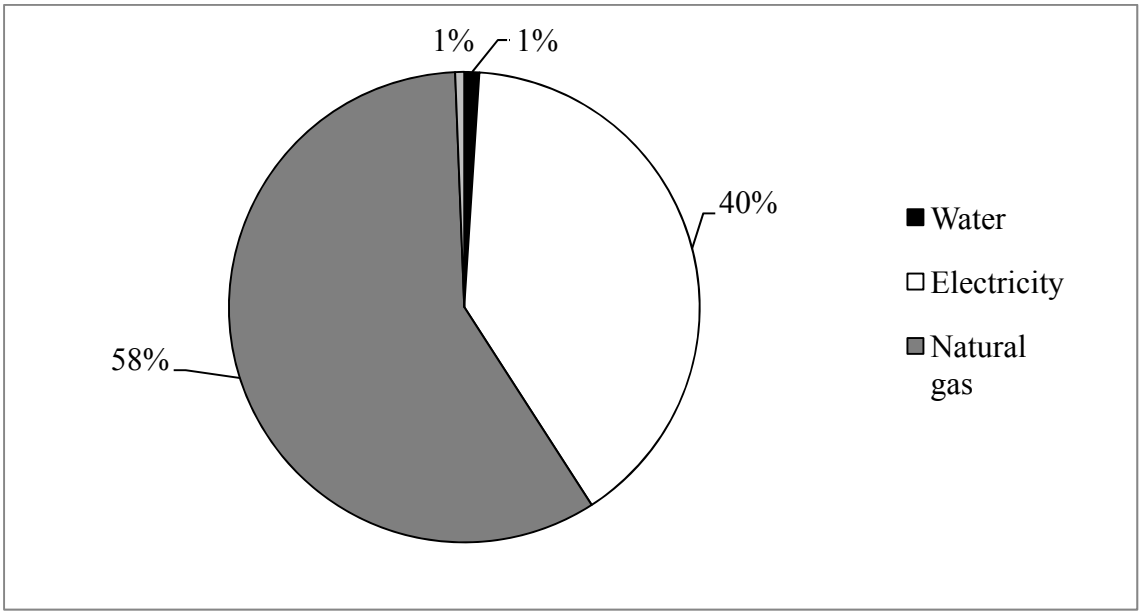

Figure 3-10: Inputs contribution to resources depletion for substrate preparation (/kg lactic acid)

Optimization of the dryer and the electrical devices would increase the environmental benefits of using potato waste starch.

In the case of corn steep liquor and transport impacts on resource depletion for media preparation (see figure 3-9), the higher energy consumption associated with these processes in the case of "PWS water" results from the direct fermentation of potato waste starch under its starchy form.

For the same lactic acid yield, the fermentation of potato starch is realized in a more diluted substrate media, due, majorly, to viscosity and lactic acid rate constraints. This results in a more diluted final lactic acid concentration at the end of the fermentation batch. Since both scenarios assumed the same initial concentration of corn steep liquor solids, the quantity of corn steep liquor required per kg of lactic acid is therefore higher for the "PWS water" scenario, and so is the required transport. From these observations, it might be interesting to hydrolyze the residue prior to its fermentation, to reduce the batch volume per unit of lactic acid. This would decrease, among others, the vapor and corn steep liquor utilization per kg of lactic acid produced.

Similar conclusions can be drawn for the climate change impact assessment. Saturated steam production is again the most contributive elementary process in the media pre-heating and purification steps. The burning of natural gas and heavy oils for its production leads to the formation of carbon dioxide and methane. Neglecting the output of gypsum, the climate change impact assessment shows that more than 98% wt CO_2 eq. in the purification step comes from carbon dioxide fossil emissions. In fact, 87% of the CO_2 eq. comes from saturated steam (see Figure 3-11).

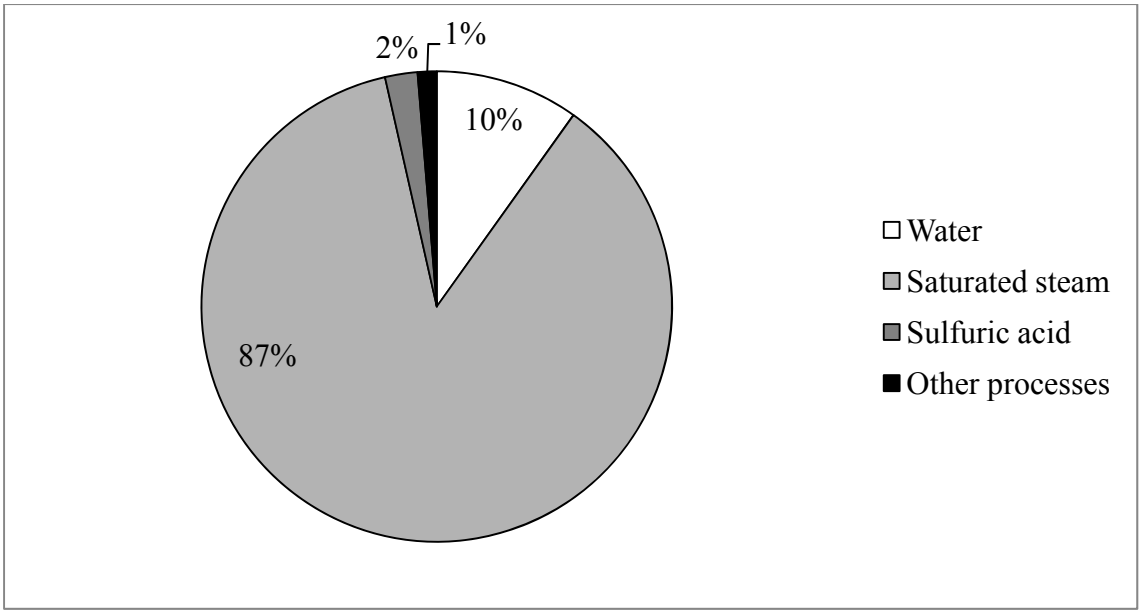

Figure 3-11: CO_2 emissions percentage for each processes of the scenario "PWS water", for the purification step (/kg lactic acid; without gypsum saving)

Media preparation is more detrimental to ecosystem quality in the case of the "PWS water" scenario (see Figure 3-12; "PWS water": 1.8 pdf·m^2·yr/kg lactic acid; "Corn": 1.1 pdf·m^2·yr/kg lactic acid). This result is again counter intuitive, since a media made partly from a waste would be expected to have less impact on the ecosystem than one made from a cultivation known for its intensive use of land and fertilizers. However, the majority of the impact comes from the corn steep liquor input and not the substrate preparation.

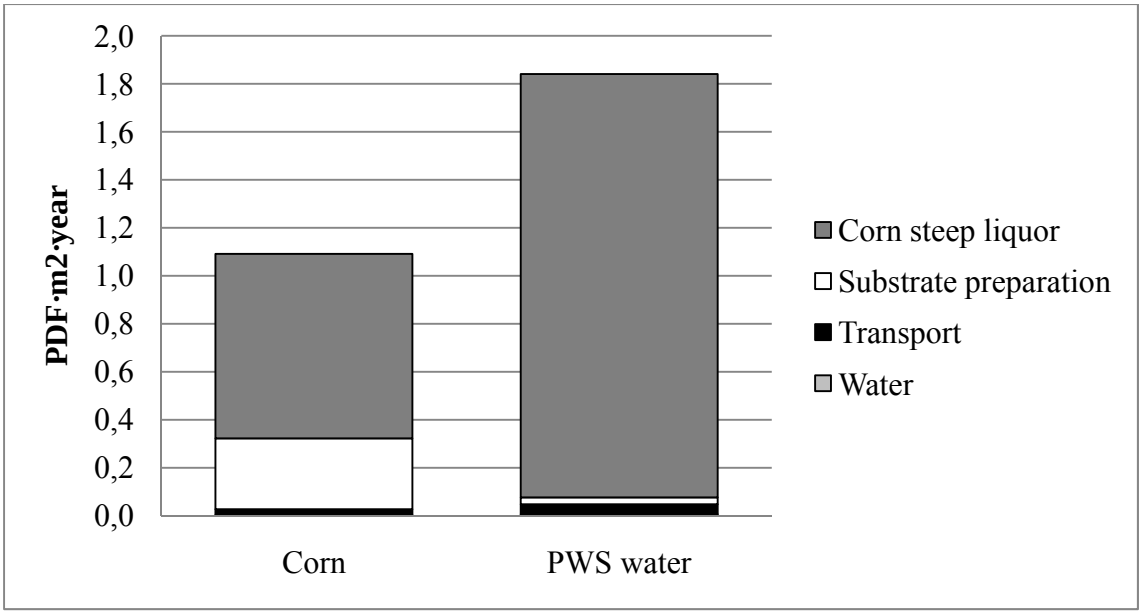

Figure 3-12: Media preparation processes contribution on ecosystem (/kg lactic acid)

Ecosystem quality damage of corn steep liquor is dominated by land occupation. In fact, the energy and emissions of corn steep liquor are associated with soybean meal, following the system expansion. Due to the lower yield of soybean cultivation and its resulting higher land occupancy, soybean is a culture with higher impacts on the ecosystem than corn and it is even more obvious in case of the land occupation mid-point category: 3.7 pdf·m^2·yr/ kg of soybean meal compared to 0.84 pdf·m^2·yr/kg of corn. Since dextrose does not displace a soybean product but its co-products do, its results in higher impacts for corn steep liquor utilization than dextrose per kg.

As mentioned before, the input of corn steep liquor per kg of lactic acid produced is higher in the case of the "PWS water" scenario because of the lower final lactic acid concentration at the exit of the fermentor. Therefore, this scenario is penalized on land occupation because of the corn steep liquor process. In the case of the purification and pre-heating steps, the input of cooling water, which is more than three times higher for the "PWS water" scenario, is also partly related to the lower final lactic acid concentration, and has, above all, impacts on the aquatic ecosystem, especially with aluminum emissions in water, coming from the life cycle of water infrastructures.

As it has been the case for each impact category, the media pre-heating and purification steps of the “PWS water” scenario is detrimental to human health compared to the “Corn” scenario. In the case of the purification step, the elementary processes that contribute the most to this end-point category are the sulphuric acid and steam production, primarily by their resulting respiratory inorganic emissions. Steam production and sulphuric acid synthesis lead to the emissions of, among others, particulates, sulphur dioxide and nitrogen oxides, since they are high energy demanding productions. In the case of the media preparation step, the lactic acid production from corn is unfavorable to human health. Human health impacts are dominated by ammonia emissions from the fertilization of the corn culture.

From these results interpretation, it can be concluded that the “PWS water” scenario, as it is currently designed, is not interesting for lactic acid production, from an environmental point of view. Sensitive points to its ecobalance, such as energy consumption (natural gas and steam), were underlined. Following these observations, different energy optimization options will be evaluated, through an “Improvement potential” analysis. Transportation distances and the method of allocation will be part of a sensitivity analysis.

Improvement potential

The scenario variants studied for the "PWS water" system are presented in Table 3-7:

Table 3-7: Variants studied for the "PWS water" scenario

Name	***Method for gelatinization***	***Residue concentration energy consumption***	***Number of effects in the evaporator***
Optimized	Continuous gelatinization	Optimized	3
Optimized + 6 effects	Continuous gelatinization	Optimized	6

The variant named "Optimized" is the one in which some partial energy optimizations have been performed on the gelatinization and the residue concentration processes, which are specific steps to the "PWS water" scenario. Gelatinization is realized with a similar configuration as the one used in continuous sterilizers, in which the outlet liquid (hot) preheats the inlet liquid (cold), through an external heat exchanger. A detailed description of this continuous gelatinization method is presented in the Appendix E. In the case of the residue concentration step, the energy consumption is reduced by operating the starch dryer with combustion gas of a frying oil boiler from the potato chip process. This will replace completely the natural gas input and will be implanted this year (2010) at the partner company. The reference flows obtained following these modifications are presented in Tables 3-8 to 3-10 (modified reference flows are in bold):

Table 3-8: Inputs and outputs for the synthesis of lactic acid via the "PWS water optimized" scenario variant, per kg of lactic acid at 88% wt produced (neglecting final impurities)

Step	***Input***	***Value***	***Output***	***Value***
Media preparation	Tap water (15°C)	24 kg	Potato peel	Unknown
	PWS (humid)*	1.1 kg		
	CSL	2.2 kg		
	Transport	1.0 tkm		
Media pre-heating	**Saturated steam (121°C, 1.1 bar)**	**1.7 kg**		
	Cooling water	**65 kg**		
Fermentation	Calcium hydroxide	0.37 kg		
	Cooling water	3.8 kg		
	Electricity	0.011 kWh		
	Transport	0.11 tkm		
Purification	Sulphuric acid	0.48 kg	Gypsum (pure, dry weight)	0.67 kg
	Saturated steam	10 kg	Sludge	1.0 kg
	Cooling water	870 kg	Wastewater	24 kg
	Transport	0.25 tkm	Lactic acid (88% wt) + impurities	1.0 kg

Table 3-9: Inputs and outputs for the concentration of potato wastewater per ton of potato waste starch (13% humidity), for the "PWS water optimized" scenario variant

Inputs	***Unit***	***Value***	***Output***	***Unit***	***Value***
Potato wastewater (1)	t	Unknown	Potato waste starch (humid)	t	1.0
Electricity	kWh	3.4E+02	Wastewater from (1)	t	Unknown
Tap Water (2)	t	16	Wastewater from (2)	t	16
Natural gas	**GJ**	**0**			

For the "PWS water optimized + 6 effects" scenario variant, an evaporator with six effects instead of three will be substituted in the previous variant. It assumes that this common step to both systems (concentration of lactic acid) would beneficiate of a higher energy saving per kilogram of water evaporated for the potato waste scenario.

Table 3-10: Inputs and outputs for the synthesis of lactic acid via the "PWS water optimized + 6 effects" scenario variant, per kg of lactic acid at 88% wt produced (neglecting final impurities)

Step	***Input***	***Value***	***Output***	***Value***
Media preparation	Tap water (15°C)	24 kg	Potato peel	Unknown
	PWS (humid)*	1.1 kg		
	CSL	2.2 kg		
	Transport	1.0 tkm		
Media pre-heating	Saturated steam (121°C, 1.1 bar)	1.7 kg		
	Cooling water	65 kg		
Fermentation	Calcium hydroxide	0.37 kg		
	Cooling water	3.8 kg		
	Electricity	0.011 kWh		
	Transport	0.11 tkm		
Purification	Sulphuric acid	0.48 kg	Gypsum (pure, dry weight)	0.67 kg
	Saturated steam	**6.2 kg**	Sludge	1.0 kg
	Cooling water	**440 kg**	Wastewater	24 kg
	Transport	0.25 tkm	Lactic acid (88% wt) + impurities	1.0 kg

✓ Results for the "PWS water optimized" scenario variant

For the comparison of this variant scenario to the "Corn" scenario, only the impacts on resources have been evaluated and are presented in Figure 3-13.

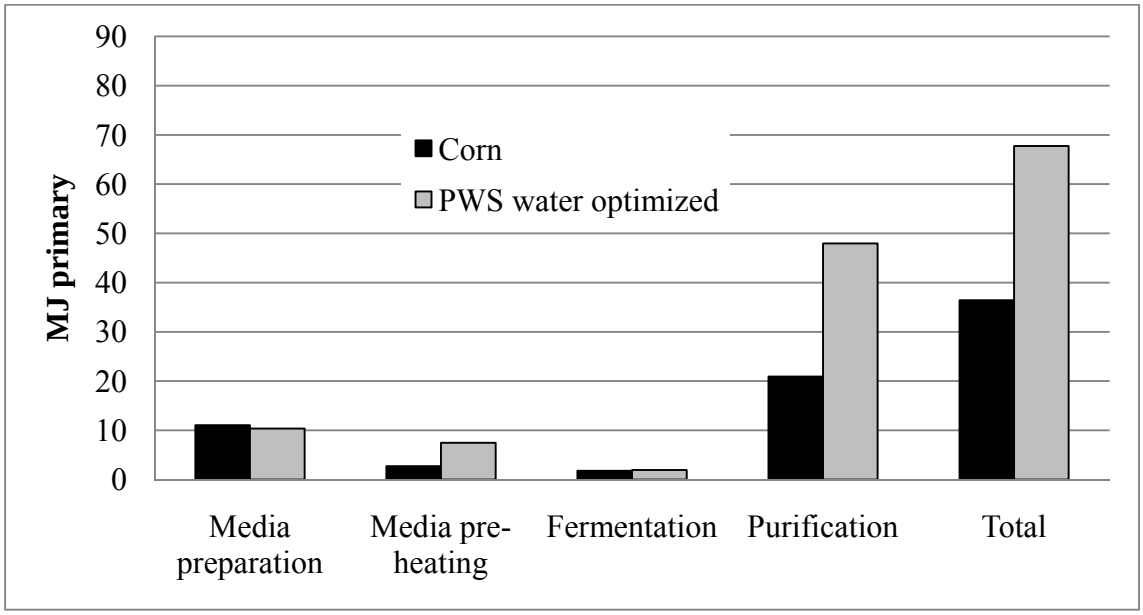

Figure 3-13: Impacts on resources, per kg of lactic acid

Following a reduction of energy consumption in the potato waste starch concentration and in gelatinization process, a reduction of resource depletion in media preparation and pre-heating can be observed. In the first case, it is related to the reduction of natural gas utilization and in the latter case, of saturated steam. The primary energy use associated with the potato waste starch concentration step has passed from 9.8 to 4.0 MJ primary/kg of potato waste starch. The primary energy use for the media preparation has passed from 16 MJ/kg of lactic acid to 10 MJ/kg, compared to 11 MJ/kg for the "Corn" scenario.

Figure 3-14 first two columns present the impacts on resource depletion by dextrose. The first column presents the impacts of dextrose if all the energy and emissions related to the corn wet milling process were allocated to it; the second, considering the energy and emissions saved following the system expansion. The contribution of corn growing and harvesting over the corn

wet milling processing (extraction of corn starch and its saccharification) is detailed. The third column presents the energy depletion associated with the potato waste starch concentration, following the energy optimization at the potato chips facility.

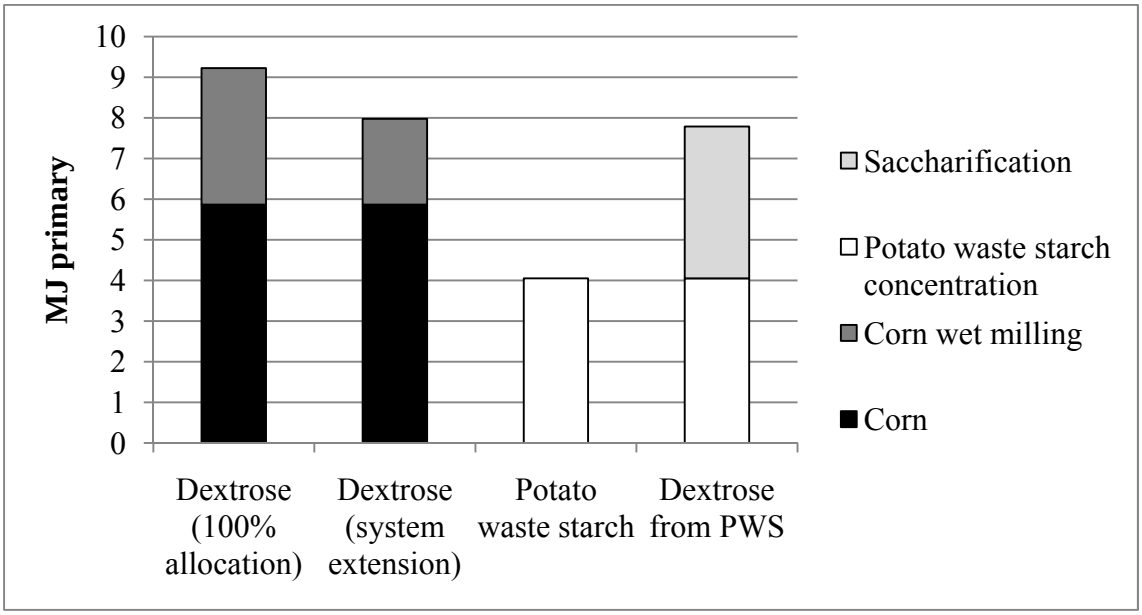

Figure 3-14: Impacts on resource depletion for the production of dextrose and potato waste starch (/kg lactic acid)

Figure 3-14 shows that the co-products system expansion is beneficial to dextrose, since more than a third of the energy consumption related to the corn wet milling process is displaced by the avoided of barley, soybean oil and soybean meal. It can be observed from this figure that even before the corn wet milling process, the needed inputs for corn growing and harvesting (5.9 MJ/kg lactic acid) have more impact on resource use than potato waste starch (4.0 MJ/kg lactic acid). In fact, corn drying requires the burning of oil and this is the major contributive process to resource depletion. Also, since corn contains about 60% starch and, therefore, of extractable sugars, its sugar yield per kg is lower than the potato residue, which is 100% starch.

The primary energy consumed during the corn wet milling step, with 100% allocation to dextrose, is 3.4 MJ/kg of lactic acid. This is in agreement with Boustead's (2001) study, which is also in the European context. The total primary energy associated with the saccharification of starch is 3.0 MJ/kg starch in the Boustead's study, assuming a yield of 0.986 kg dextrose/kg starch extracted, before any allocation method. The emissions and energy consumption related to starch extraction are also given per bushel transformed. Knowing that one bushel can produce 14.3 kg of corn starch, it can be deduced that the impact related to starch extraction is only 0.0085 MJ/kg starch. The saccharification is the heavy energy consumer step of dextrose production in a corn wet milling facility.

The last column of Figure 3-14 presents the impacts on resource depletion for the production of dextrose from potato residue. The transportation distance is assumed to be the same for potato waste starch as for corn (100 km). According to Boustead (2001), the energy consumption related to starch saccharification should not be affected by its origin, once it has been extracted and purified. The difference between the impact of the potato waste starch saccharification compared to the corn wet milling process remains in the co-product system expansion. In the case of the residue, there is no co-product produced during potato waste starch concentration.

From these interpretations, it is assumed that synthesizing dextrose from the residue has an associated primary energy of 7.8 MJ/kg, compared to 8.0 MJ/kg from corn. The potential impact of the media preparation step would be the same for both product systems, but would avoid the use of a food source and ILUC in the case of the starch residue. All environmental impact of the subsequent steps would be the same. The reasons for such similarity between a media prepared from a waste to one from a food source, and potential solutions will be presented in the general discussion section (Chapter 4).

- ✓ "PWS water optimized + 6 effects" scenario

The impact in each endpoint category for this variant is compared to the "Corn" scenario in Figures 3-15 to 3-18:

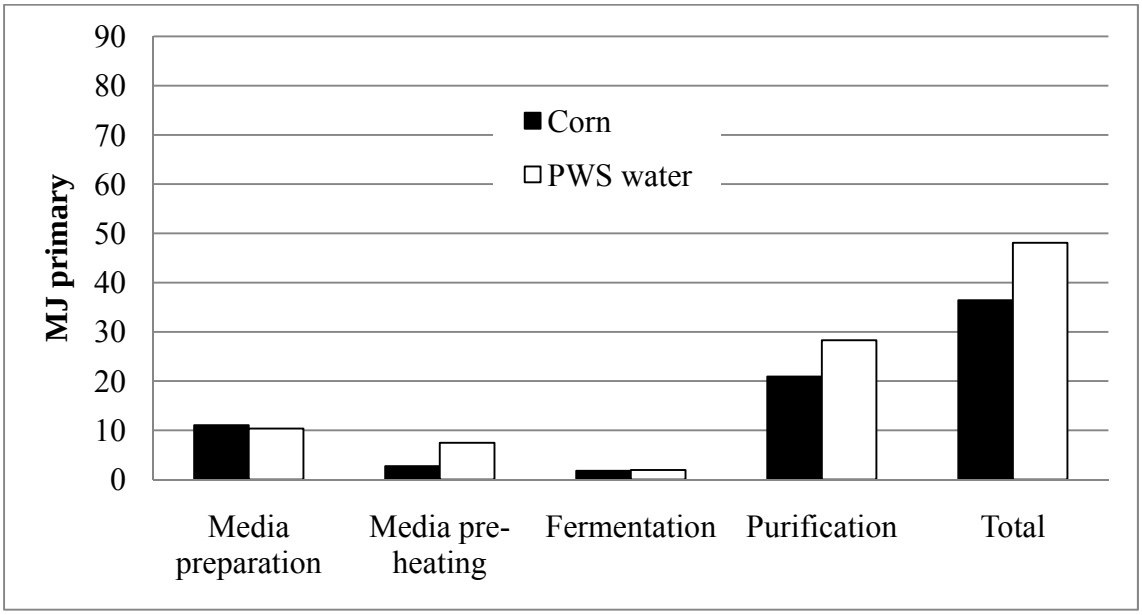

Figure 3-15: Impacts on resources, per kg of lactic acid

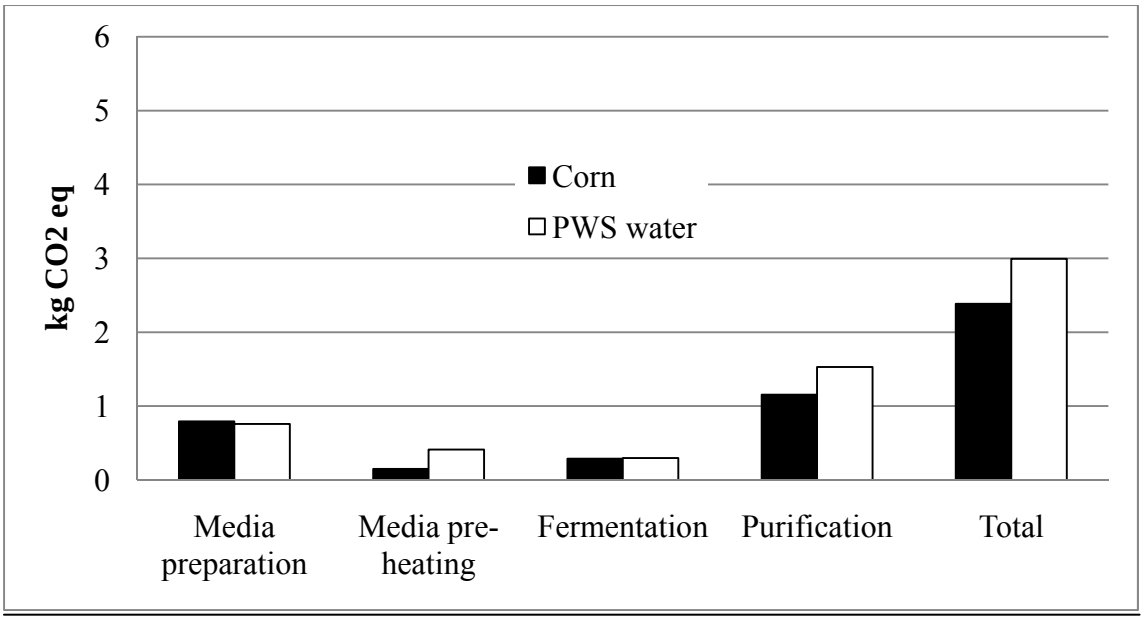

Figure 3-16: Impacts on climate change, per kg of lactic acid

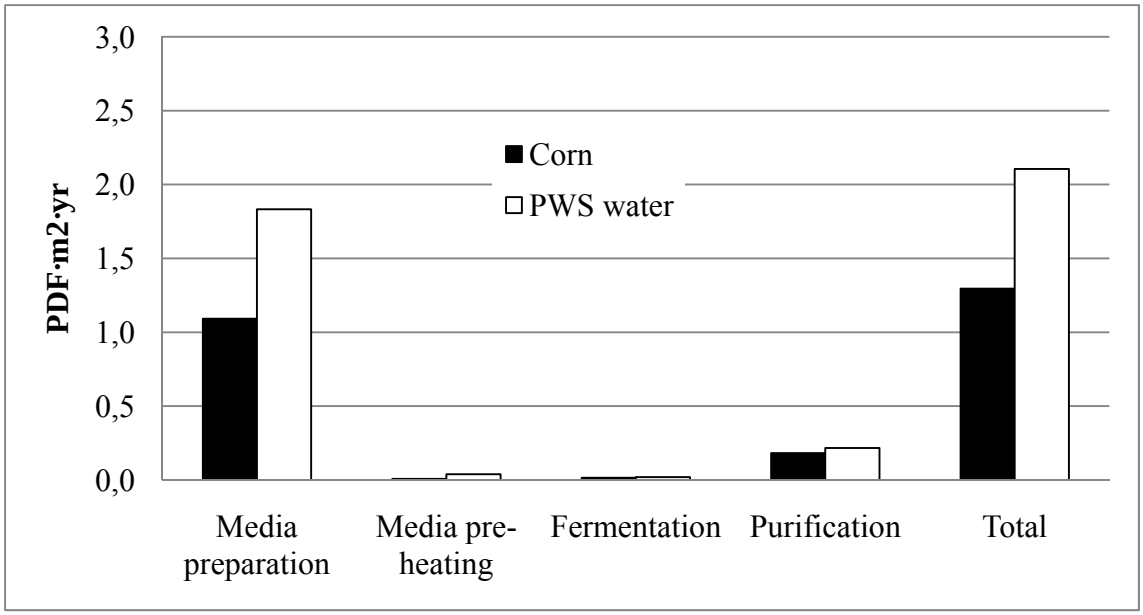

Figure 3-17: Impacts on ecosystem quality, per kg of lactic acid

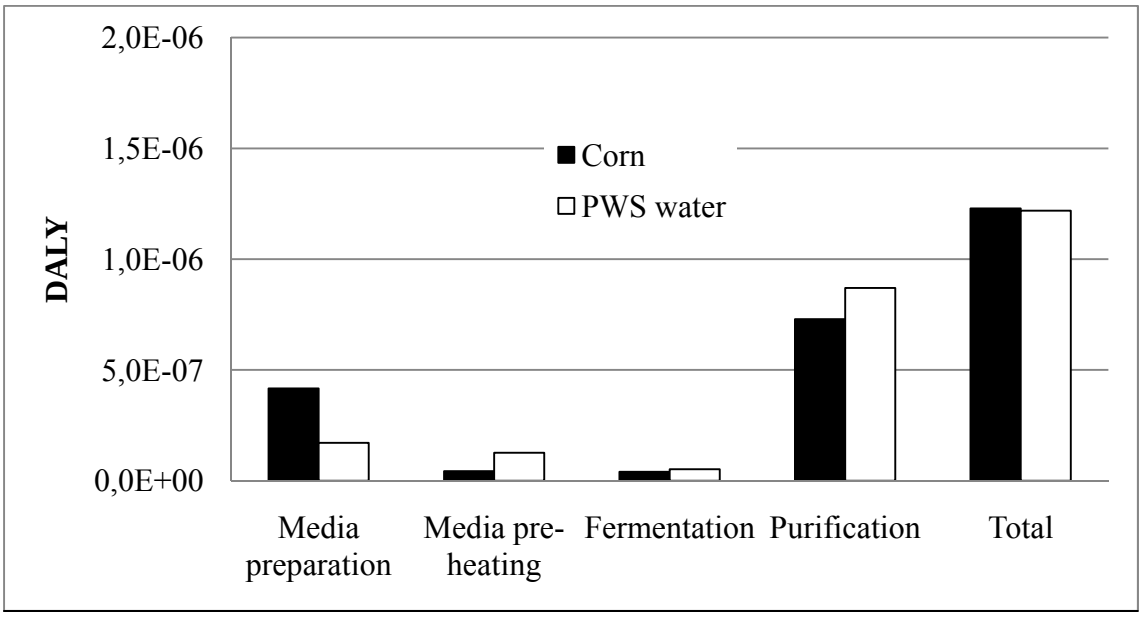

Figure 3-18: Impacts on human health, per kg of lactic acid

It can be observed from Figures 3-15 to 3-18 that the impacts of purification in each damage category has been lowered for the “PWS water” scenario. However, it remains up to about 35% higher than the “Corn” scenario for the end-point category studied.

The addition of effects in an evaporator decreases its steam demand, but also its cooling water demand since more of the evaporated water is condensed into the unit effects. In the case of resource depletion, this partly compensates the high saturated steam consumption of the potato waste starch process, due to its more diluted form in the media for the same yield reached. By doubling the number of effects in the case of the “PWS water” scenario, impact of purification on resource depletion becomes closer to the one of the “Corn” scenario.

As mentioned before, the impact on climate change is closely related to the impact on resources, due to the inputs of steam. As for resource depletion, the impact of purification on climate change is more similar to the one of the “Corn” scenario, following the addition of 3 effects for the “PWS water” scenario.

The reduction of steam consumption in “PWS water” scenario has also an effect on ecosystem quality. The impacts of purification are also lowered. This is mostly induced by the cooling water reduction, the first contributive process in the purification step of previous scenarios. Steam reduction has also a beneficial impact on the human health category for the “PWS water” purification step. In fact, more than half of the impact on human health by steam results from the burning of heavy oils. If steam was produced from natural gas only, its impact on human health and ecosystem would be reduced by more than 50% (see Figure 3-19)

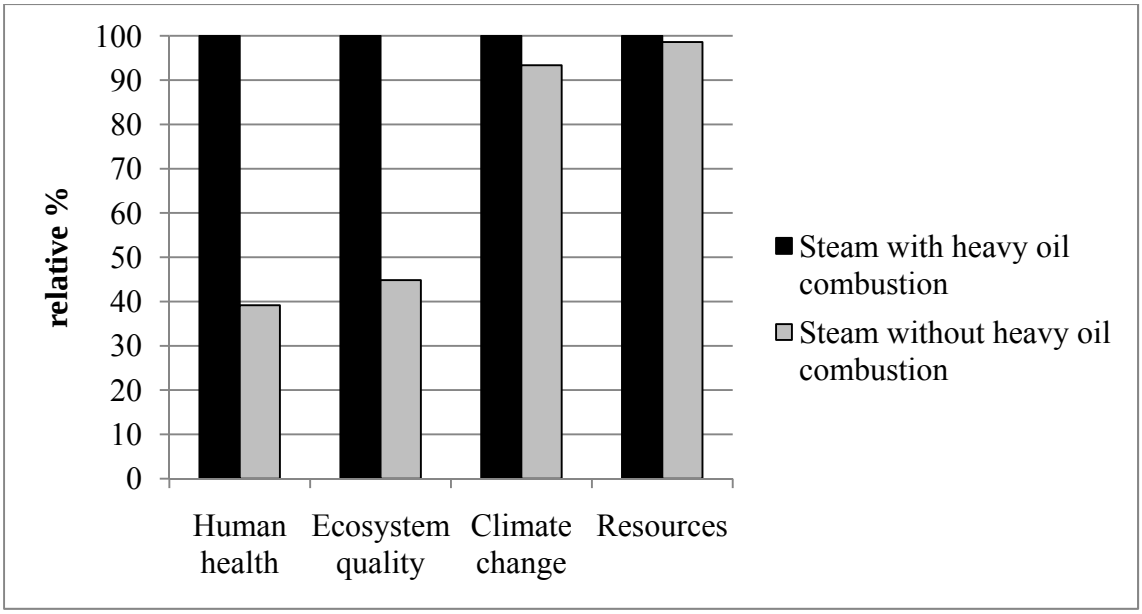

Figure 3-19: Relative percentage of steam impacts, with and witout the burning of heavy oils (/kg steam)

This fuel scenario is probably more realistic, since a plant normally uses a single source of energy. Comparatively, the Ecoinvent data is average over many plants, including older ones. This aspect should be considered in future studies. Table 3-11 presents the final impact of both scenarios for the end-point damage category:

Table 3-11: End-point impact values for both scenario (/kg lactic acid)

End-point category	***"Corn" scenario***	***"PWS water optimized + 6 effects" scenario***
Resource depletion (MJ primary)	36	48
Climate change (kg CO_2 eq.)	2.4	3.0
Ecosystem quality (pdf·m^2·yr)	1.3	2.1
Human health (DALY)	1.2E-06	1.2E-06

This variant scenario has impact which tends to reach those of the “Corn” scenario for the climate change and the resource depletion damage category. If the number of effects at the evaporator is increased again, it should be expected that both scenario could have the same impact in these categories, since the burning of natural gas for steam production is the major contributor. In the case of human health damage ctaegory, the impacts are identical. In the case of ecosystem quality, there is a difference of more than 60% between both scenarios. It results from the higher input of corn steep liquor per kg of lactic acid for the “PWS water optimized + 6 effects” scenario. This has repercussions of land occupation, as previously discussed.

The “PWS water” scenario results cannot be compared to any LCA study since this study is the first of its kind. In the case of “Corn” scenario, Bohlmann (2004) has performed an LCA for a similar lactic acid production process, based on the same purification units (gypsum formation and multi-effect evaporation) and with the help of a Superpro designer simulation. The primary energy consumption of their lactic acid simulation, with a 3 effects evaporator, was 30.4 MJ/kg of lactic acid (88 wt%). This value is about 20% lower than the one of this present study. This is partly explained by the different paramters set: the author assumed a yield of 87% and a final calcium lactate of 12 wt% (fermentation temperature: 50°C). In Bohlmann’s LCA, water consumption and transport were not evaluated. However, impurities purification and pumps electrical consumption was taken into account in the simulation, and so in the LCA. By comparing the different reference flows with the help of private discussions with Mr. Bohlmann, it was found that corn steep liquor and electrical input value were highly different between both studies. In the case of electrical consumption, this is explained by the complete process simulation which was realized in Bohlmann’s LCA, which take into account pumping, neglected in our study. Only 0.045 kg of corn steep solids/kg of lactic acid were used in Bohlmann’s study and this value is considered as really low and probably questionable, knowing the complex nutrient needs of lactic acid bacteria. Bohlmann’s study was also representing the North American context, and not the European one, which differs, among others, in electricity mix ratio, transport and technologies.

In the case of Natureworks LCA, even if the process description of their last published LCA was used as a reference for this study, only aggregated values are presented in their results and so the impacts of lactic acid synthesis cannot be compared. However, the Natureworks study of 2003 presents aggregated data for the primary energy consumption for all processing steps. Since corn growing and harvesting and its saccharification through the corn wet milling process are mature processes, the associated primary energy consumption should not have varied a lot through the years. They represent respectively 4.9 MJ (corn growing and harvesting) primary and 9.4 MJ (corn wet milling) per kg of polylactide produced. Since the literature reveals that about 0.7 kg of polylactide is obtained from 1 kg of starch (NCGA, 2009), and nearly 1 kg of dextrose is produced from 1 kg if starch, the energy associated with dextrose is, including transport, 10.3 MJ/kg dextrose. This value is similar to the energy associated with the dextrose$^{100\%}$ of our study. NatureWorks' LCA also represented the North American context.

The potato starch process variant "optimized + 6 effects" will be used for further sensitivity analysis.

Sensitivity analysis

✓ **Method of allocation for the corn wet milling co-products**

In this part of the sensitivity analysis, two methods of allocation for the co-products of the corn wet milling process are compared. Figure 3-20 presents the primary energy consumption associated with the media preparation step for the "Corn" scenario and the "PWS water" scenario; Figure 3-21, the impact on ecosystem quality.

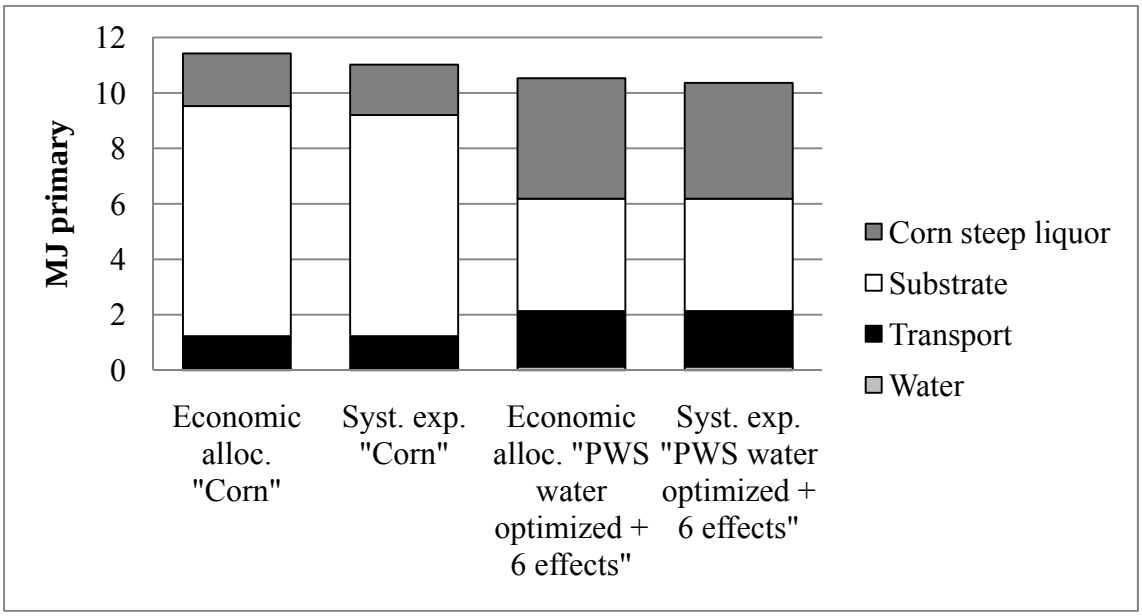

Figure 3-20: Impact on resources by the media preparation as a function of allocation method

Primary energy consumption is not influenced if an economic allocation, based on the selling prices of dextrose and its co-products (corn gluten meal, corn gluten feed, corn oil and corn steep liquor), is used instead of a system expansion.

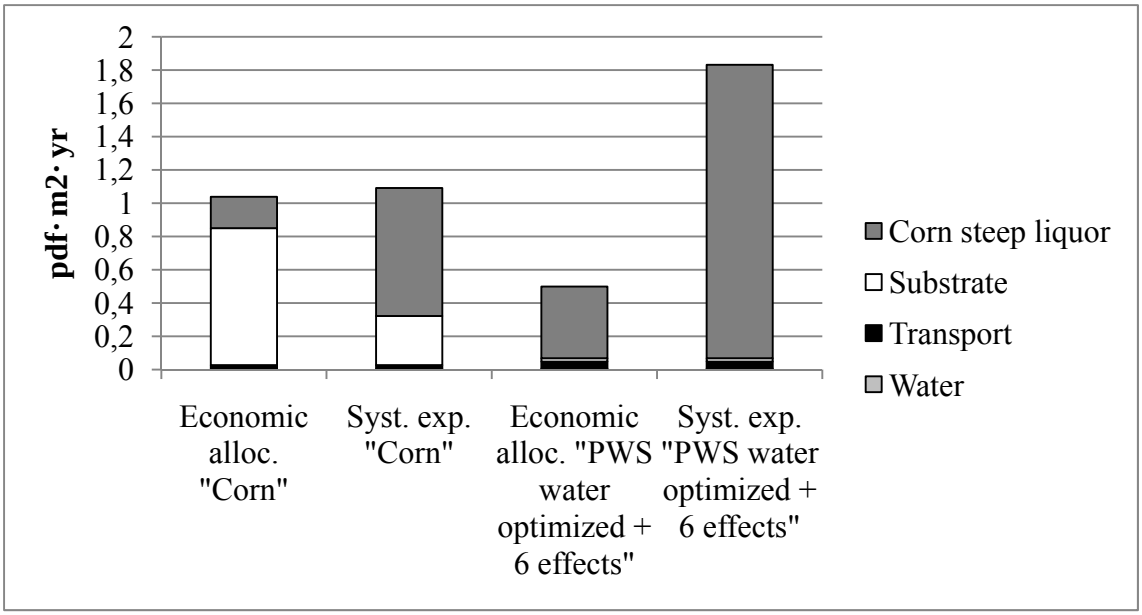

Figure 3-21: Impact on the ecosystem by the media preparation as a function of allocation method

For the ecosystem impact category, the choice of the allocation method is significant on results. It increases the impact of the dextrose, and decrease the one of the corn steep liquor. Therefore, the "PWS water" scenario is advantaged by the economical allocation method.

- ✓ **Transport**

In this part of the sensitivity analysis, the transportation distances were doubled for both scenarios. For most inputs, distances were increased to 600 km, except for the sludge and corn; the distance to reach the sanitary landfill and the corn wet milling facility was doubled to 200 km. As a result, the primary energy consumption was only increased by less than 7%, for both scenarios. Increases are also small in other impact categories. This demonstrates that transport is a not a significant process in these systems.

CHAPTER 4 GENERAL DISCUSSION

Laboratory experiments have confirmed the microbial feasibility of producing lactic acid from the direct fermentation of potato waste starch with *L. amylophilus NRRL B-4437*. However, the important variability has demonstrated the complex behavior of starch and the variety of physicochemical properties that can be associated with different types of starch. The best example was the different final viscosity and digestibility of potato waste starch media obtained after sterilization, compared to commercial starch. This has proven that any similar behavior/digestibility cannot be guaranteed between two starches of different origins, even if they come from the same kind of plant (potato). It is therefore obvious to well understand wthe behavior of a starch during heating and in the presence of other medium constituents, but also the associated digestibility with the bacteria studied. Appropriate experiments to characterize the starch behavior are essential before its use in industrial lactic acid production.

As underlined in the state-of-art section, only a few publications exist on *L. amylophilus*, and specific publications on the *NRRL B-4437* strain are rare. Optimal conditions for lactic acid production were studied to a limited extent. Any published studies with *L. amylophilus NRRL B-4437* do not present experiments on preculture or a study on starch gelatinization methods (Pierre Mercier, 1990; P. Mercier, et al., 1992; Nakamura, et al., 1979; Pompeyo, et al., 1993). P. Mercier et al. (1992) have tested heating pretreatment methods for a corn starch medium that include acid or commercial enzyme addition. This type of pretreatment, which partly hydrolyzes starch and increase the enzyme digestion rate and so the lactic acid production, was excluded from our LCA study. This Lactobacilli strain (*NRRL B-4437)* is not well-documented and should be more studied before evaluating its industrial potential, as it was done for largely employed bacteria of this genius (ex.: *L. delbrueckii*). Also, other strains might be more adapted to potato starch fermentation. For example, *L. amylophilus GV6* produces an amylase but also an amylopullulanase, which makes this strain more adapted to starch digestion. The use of this strain and further genetic modifications could improve *L. amylophilus* lactic acid rates and yields on potato starch.

The laboratory experiments did not only demonstrate the feasibility of producing lactic acid by the direct fermentation of potato waste starch with *L. amylophilus*, which was the decisive criterion to realize this LCA, but they have also helped, with the literature review, to understand the challenges and limitations of directly fermenting starch and the required information for future laboratory experiments. Based on laboratory observations, literature and bioengineer's point of view, it was possible to set-up for the LCA realistic and conservative fermentation parameters, despite the inexistence of any industrial scale production of lactic acid from potato waste starch. This LCA study, comparing lactic acid produced from potato wastewater and from corn, had two major differences between the scenarios studied:

- Type of substrate employed and its origin: foodstuff versus a waste;
- The direct fermentation of starch compared to its complete hydrolyzed-form;

In the case of the media preparation, potato waste starch has an environmental potential comparable to dextrose from corn, if same lactic acid yields are reached. It has been demonstrated, for the process conditions fixed in chapter 2, that the residue has less impact on resource depletion than dextrose from corn per kg of lactic acid produced (4,0 MJ primary/kg lactic acid versus 8,0 MJ primary/kg lactic acid). However, as underlined in the interpretation, a major difference in resource depletion and climate change impacts was expected between both substrate production. In fact, the benefits of using a waste were cancelled by the large amount of energy required to concentrate it. Two aspects do not favour the energy consumption during the potato waste starch concentration: the low concentration of potato waste starch in wastewater and the technological immaturity (13 year's old versus more than 150 year's old for the corn wet milling process).

Since potato waste starch is highly diluted in wastewater (< 1 wt%), almost all the pumping energy is used to displace water and to drive the separation process with hydrocyclones. This leads to a low potato waste starch production rate and to high energy flows per kg of residue produced, which is not expected for pumping; pumping is generally not significant. Before natural gas reduction (variant "optimized"), the primary energy consumption of pumps and

motors was only about 30% lower than the one of the dryer. Efforts for reducing the electrical consumption should be performed in the coming years to improve the potato waste starch ecoprofile. This could be realized by pre-concentrating the wastewater in the process upstream. Lower electrical consumption could be also realized by privileging mechanical separations with lower operation energy, such as filtration (ex.: vacuum or pressure filters). Also, the introduction of grinded rejected potatoes should come further in the concentration process. With immaturity, there is also a risk that the process equipment is oversized, due to an incorrect conception or to an overestimated production rate. The potato waste starch concentration in wastewater is variable, since it fluctuates with the potato cultivar, the potato age and the quantity of potato rejected. It would be interesting for further ecoprofile analysis to have data average on a multi-year period.

This study has raised problems related to the direct fermentation of potato waste starch, which are its complex properties and behavior. As previously mentionned, a medium made of starch will have a viscosity and a degree of digestibility associated to the preparation method and the substrate concentration. These complexe properties should be considered, since the enzyme production, which enables the assimilation of starch, relies on the growth and survival of the inoculated culture. The enzymes effectiveness can also be restricted by culture condition limitations, such as temperature, to not reduce growth rate. This may explain, in part, why the industry is still using simple sugars, produced from starch saccharified by concentrated and specialized enzymes.

In this study, the savings from not saccharifying the substrate in the case of “PWS water” scenario were overcome by a lower lactic acid concentration at the exit of the fermentor, which results in a higher steam consumption in the purification step. It has been demonstrated that this could be partly overcomed by increasing the number of effects in the evaporator. Other effects could be added. More than ten effects evaporator can be find in the industry. More investment in capital would be needed. In the case of the gelatinization steps, according to the literature, the energy consumption could be decreased by increasing the shearing rates on the starch granules, by grinding them or by agitating the starch solution. Shear damages starch granules, which, in

turn, reduces the gelatinization temperatures and the viscosity. This could also increases the maximum temperature allowable in the heat exchanger without fouling.

In the case of the media preparation step, the higher mass of corn steep liquor needed per kg of lactic acid produced is problematic in the ecosystem quality, the resource depletion and so in the climate change categories, when considering the system expansion allocation method. To avoid this, the concentration of corn steep solids should be reduced so far as it does not compromise the cell growth and the lactic acid yield. However, experiments performed with lactic acid bacteria uses the same protein content in literature, even for higher substrate concentration. There might exist a concentration limit at which proteins can be efficiently used. But also, it might be ovestimated for the reproductibility needs of the laboratory context. Other protein sources might also be more interesting in terms of performance (i.e. lactic acid production rate) and overall ecobalance (i.e. lactic acid LCA). Surface-response methodology could be an efficient statistical method used to evaluate optimal concentration of different protenious substances. Finally, proteins could be recycled from the mechanical filtration step, but this increase the risk of contamination.

Of course, the fermentation of a more concentrated media for the "PWS water" scenario could overcome partly or totally all these environmental discrenpencies with the "Corn" scenario, if it results in a higher final lactic acid concentration. However, an increase in the starch concentration leads to a higher viscosity, which may be detrimental to the operations. Lactic acid production rates generally decrease fast at high substrate concentration, based on literature with *L. amylophilus*. Increasing the starch concentration should be evaluated with care, by optimizing the ecobalance between the benefits of having a more concentrated lactic acid media (less energy at the final purification step) and the disadvantages of having more residue not consumed (more substrate input at the media preparation). It might be needed to pre-hydrolysed the media if the viscosity cannot be at an operational and fermentable state and to improve lactic acid production rates. Pre-hydrolysis involves new inputs that would have an impact on the LCA. In any case, fermenting a highly concentrated potato starch medium requires a delicate optimization of the

media and bacterial inoculum. Finding high-performance amylolytic Lactobacilli could take time, and dextrose-fermenting lactic acid bacteria are one step ahead. Also, depending on the purification method used, an increase of calcium lactate concentration might necessitate an higher fermentation temperature, to prevent precipitation. Genetically-modified *L. amylophilus*, tolerant to higher temperature, might be necessary to prevent calcium lactate precipitation, and will lower the risk of contamination at the same time.

It was also demonstrated in this study that using dextrose from the saccharification of potato waste starch instead of corn could give, presently, the same impact for the substrate in the ressource use category, while avoiding ILUC. The residue is also economically competitive to corn starch, as it has a selling price comparable to corn starch: 0.075 US$/dry kg of potato waste starch (industrial partner selling price) versus 0.072 US/kg for corn starch (Baker, et al., 2010). The use of dextrose made from potato starch residue could be an easy short term modification to implement.

In conclusion, the “PWS water optimized + 6 effects” scenario utilizes an unoptimized corn steep liquor concentration, and further energy improvements for the substrate preparation and the lactic acid purification could be realized in the future. It should be expected that this process configuration could reach at least the same environmental impacts than the scenario “Corn”, while avoiding the use of a food source and ILUC. The environmental potential of developping such a process has been demonstrated.

CONCLUSIONS AND OUTLOOK

The aim of this study was to measure the feasibility to produce lactic acid for polylactide usage and to evaluate the environmental impacts (LCA) by the direct fermentation of an agribusiness waste, potato waste starch, with *L. amylophilus*. A life cycle assessment was conducted to compare the environmental impacts of lactic acid produced from this innovative process with a conventional one using dextrose from a foodstuff as a raw material, corn. This objective was attained by, firstly, demonstrating the feasibility of producing lactic acid from this residue with a *L. amylophilus* strain. Following these experiments, the process flowsheets and parameters were set, based on literature, experiments and general knowledge in bioengineering. Energy and mass balances were realized and the necessary references flows for the LCA were calculated. A comparative impact assessment from these reference flows was realized and has demonstrated that producing lactic acid for PLA applications could be environmentally favorable over the typical corn process after proper optimizations.

The LCA results have demonstrated the inconvenience of working with a more diluted system for the "PWS water" scenario. Simulations have demonstrated that this could be partly overcome with energy optimizations. In the case of the potato waste starch, the impact of its initial concentration step is sensitive to the electrical consumption, which should also be optimized in the future. The most optimistic scenario for lactic acid production from potato waste in this study gave total impacts of 48 MJ (resource use), 3.0 kg CO_2 eq. (global warming), 2.1 pdf·m^2·yr (ecosystem quality) and 1.2E-06 DALY (human health) per kg of lactic acid produced. These impacts tend to reach the ones of the conventional process (~ 0-30% of difference) by improving energy consumption, except for ecosystem quality (+ 60%). This latter result relies to a great extent on the corn steep liquor utilization, which has been fixed at the same solid concentration in both system media. Further investigations on starch behavior, its digestibility and also its nutrient optimum concentration are necessary to evaluate other possible improvements, such as increasing the substrate concentration or decreasing the corn steep liquor solids concentration. Following these improvements, there is a great perspective to improve the ecobalance of lactic acid produced with potato waste starch and *L. amylophilus*, but, moreover, to make it environmentally competitive to the conventional processed lactic acid. It was not underlined in the framework of

this environmental study, but the use of waste also saves corn. One kilogram of potato waste starch can save more or less 1.7 kg of corn if the same lactic acid yield is reached and avoid ILUC. Unfortunately, the experiments realized in this study and the available literature were not sufficient to establish the optimal substrate and nutrient concentrations with exactitude.

This study has also raised the difficulty of accessing industrial data. Life cycle assessment on industrial bio-chemical processes is still in its infancy. Moreover, the competition between businesses is strong, since there are only a few lactic acid and PLA industries. Therefore, data diffusion is strictly limited. For these reasons, it has to be kept in mind that the parameters and processes chosen in this study for lactic acid production from corn have been improved, as revealed by NatureWorks website. However, the details of these optimizations, which focus on lactic acid fermentation and purification, have not been published yet. Parameters were set with the help of literature and patents, but might not represent the actual ones. The lactic acid process from corn in our study probably represents the technology which dates back to a couple years ago. Nevertheless, since this study was comparative, the necessity was to consider the same technology used for each process compared, allowing a fair comparison, and not to give present impacts. This study has raised the sensitive points (ex.: vapor use) which should be considered with care with further research for optimizing the polylactide ecobalance. An economic assessment of a this new process configuration should be realized in the future, to demonstrate its economic feasability.

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APPENDIX A – Glossary

Definition are based on these references: Thomas et al. (1999), Myllärinen (2002), Swinkels (1985), Olkku et al. (1978), S. S. Shamekh (2002)

Starch

Starch, the main reserve food of plants, is a polymer of glucose and exists under the form of granules at its native state. Starches can contain two types of glucose polymers, made of alpha glucosidic bonds: amylose and amylopectin. The ratio between amylose and amylopectin and the granule size depends on the origin of starch. Most starches contain both polymer types. Amylose is a linear polymer having a few branches ($M \approx 500\,000$ g/mol). Amylopectin is a branched polymer (10^7-10^9 g/mol). Crystallinity of starch is mostly due to amylopectin.

Gelatinization

Native starch is not soluble in cold water. Absorption of water is therefore limited and reversible. At a certain temperature, called the initial gelatinization temperature, some starch granules start to swell irreversibly and, simultaneously, to lose their birefringence due to semi-crystalline structure disruption. Since all granules do not begin to swell irreversibly at the same temperature, starch granules start to gelatinize in a narrow range of temperatures, called the "gelatinization temperatures". In the case of potato starch, it is around 58-65°C; for dent corn, 62-80°C.

At the beginning, the granules absorb water through the amorphous regions. With heating, granules swell many times their original size, crystalline structures are disrupted and amylose polymers diffuse first in the surrounding media. This disruption of molecular order can be followed by measuring an endothermic transition peak on a Differential Scanning Calorimetry (DSC) scan.

With granule swelling and macromolecules leaching, the viscosity of the media increases and, if water is not limiting, will reach a peak, where the granules are at their largest swollen volume. At this state, granules are highly susceptible to mechanical and thermal breaking. Further heating or agitation will lead to granule fragmentation and additional polymer dissolution, until the maximal dispersion is reached. The viscosity of the media is partly reduced during granules disruption.

The point at which gelatinization ends is variable from one researcher's definition to another, since it depends on the method used to monitor this phenomenon. In the case of this study, "gelatinization" regroups all the previously mentioned irreversible changes occurring during the heating of a starch solution, as define by Swinkels (1985). Figure A-1 summarizes the physical and viscosity changes occurring during the gelatinization of a starch solution (with an excess of water).

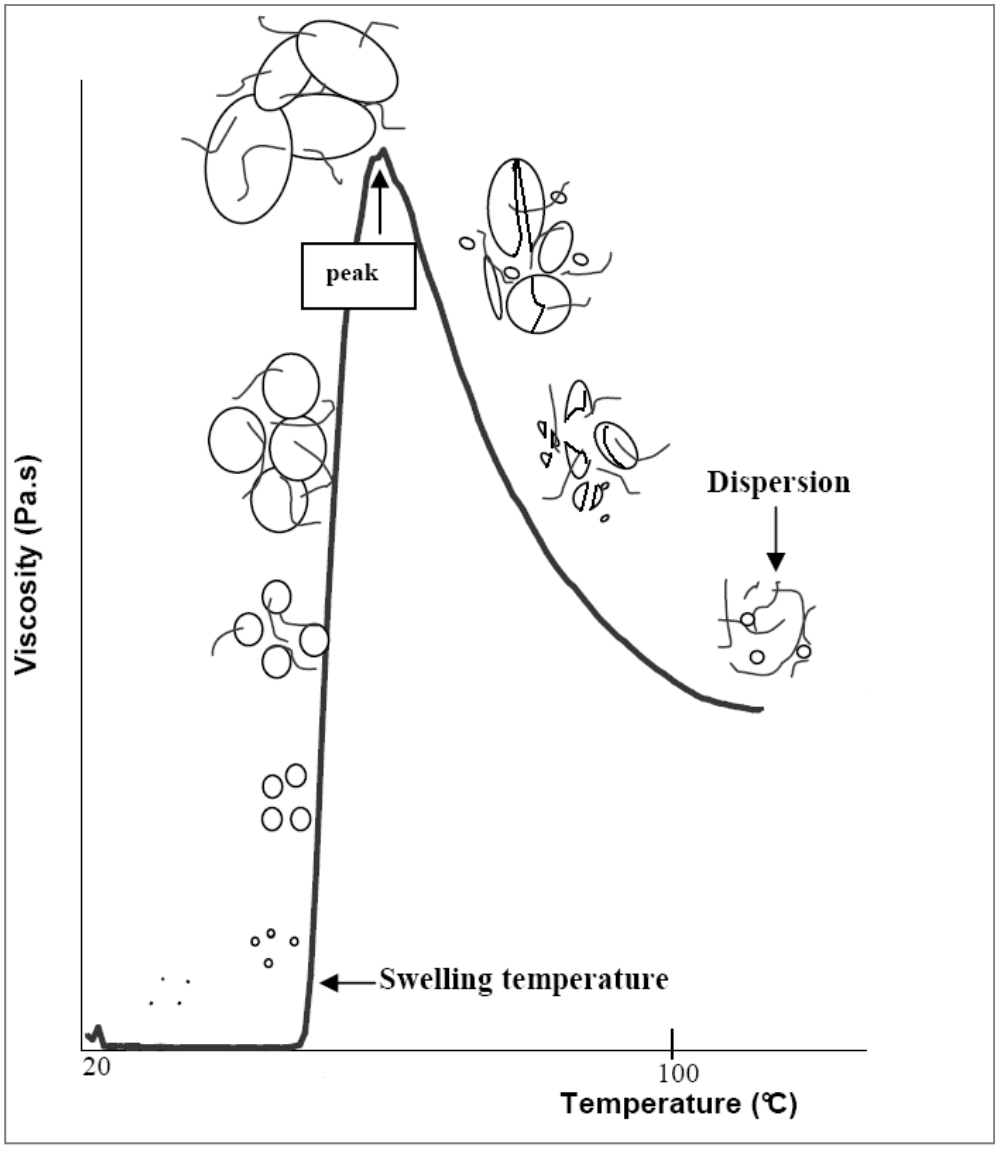


Figure A-1: Schematic representation of granular changes in relationship to viscosity, adapted from Desse (2008)

Retrogradation

Retrogradation, also called "set-back", is a process which occurs when starch chains reassociate. When a gelatinized starch solution, also called "starch paste", is allowed to stand and cool, solubilized starch polymers and granules fragments tend to reassociate and form a crystalline structure. This increases the viscosity of the starch paste. If the starch dispersion is concentrated enough, an elastic gel will form. Retrogradation occurs faster with amylose chains compared to amylopectin chains. The viscosity changes upon gelatinization and cooling is commonly followed with a Brabender visco-amylograph. A typical Brabender curve for wheat starch is shown in Figure A-2.

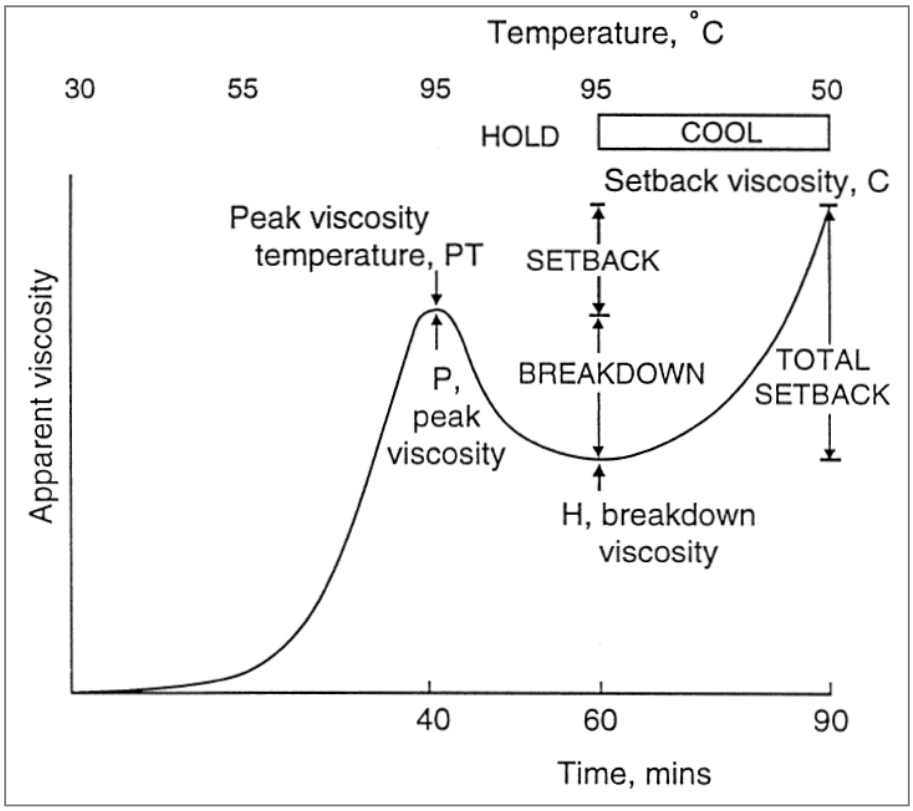
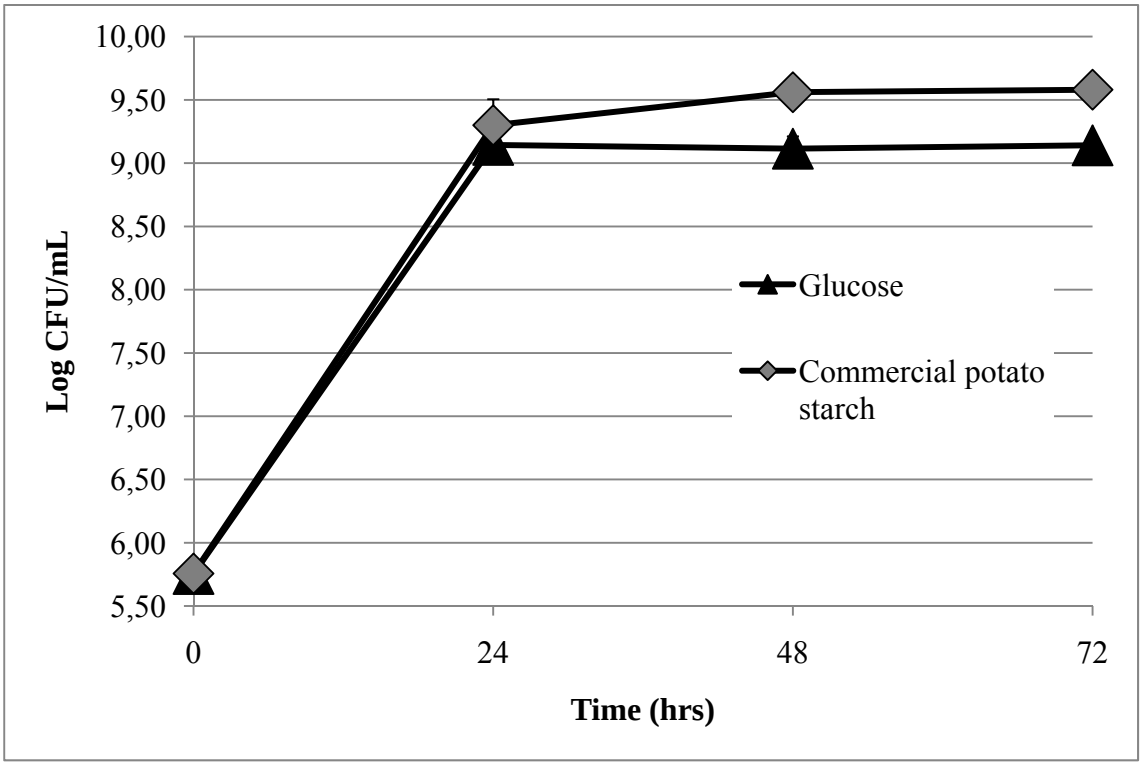
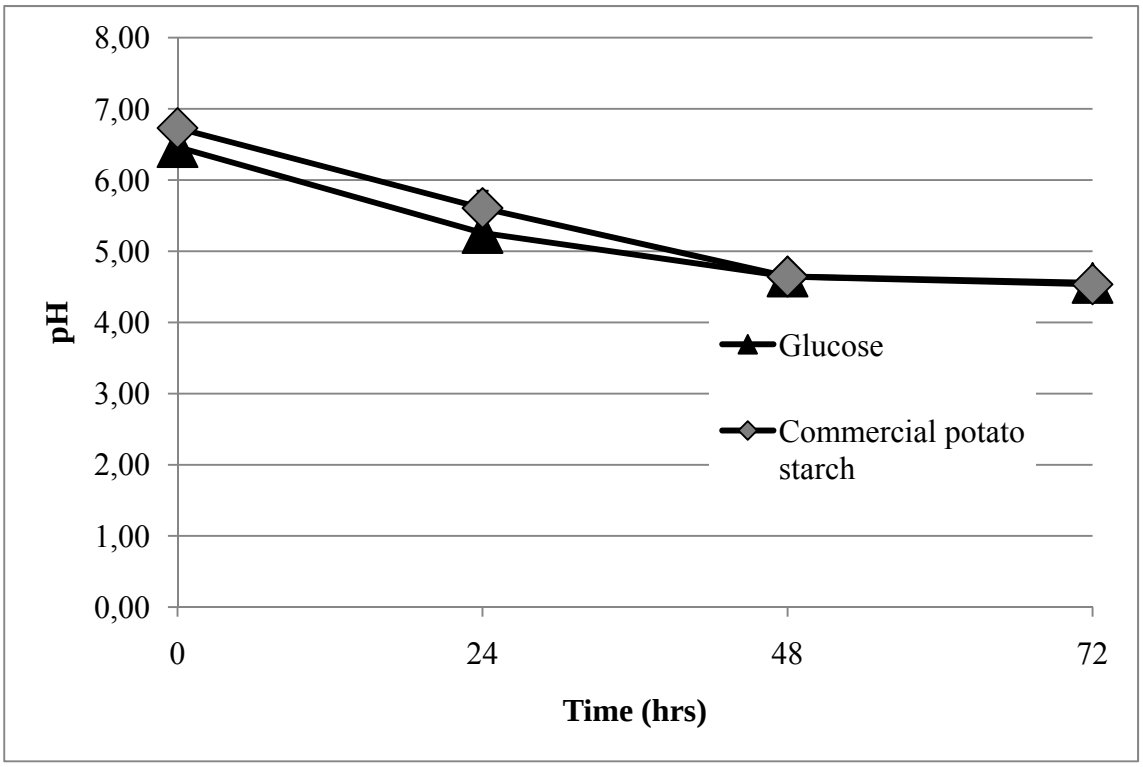


Figure A-2: A pasting cycle curve, typical of wheat starch, adapted from Abd Karim et. Al (2000)

APPENDIX B – Preculture tests



FigureB-1: Growth of precultures during time for different MRS medium



FigureB-2: pH of precultures during time for different MRS medium

APPENDIX C – Corn steep liquor specifications

Table C-1: Partial composition of corn steep liquor (pH = 4.2, total solids: 54 wt%) (Kampen, 1997)

	% dry basis
Ash (oxide)	17
Crude protein (Nitrogen x 6.25) % dry basis	47
Fat	0.4
Total acids as lactic acid	2.6
Nitrogen	7.5
Phytic acid	7.8
Reducing sugars as glucose	2.5

Table C-2: Characteristics of corn steep liquor at 45 g/L

pH	5.5
Nitrogen content (g/L)	3.1

Table C-3: MRS nitrogen content (laboratory mesurements)

Nitrogen source	***Nitrogen content (%)***	***Concentration in MRS (g/L)***	***Nitrogen concentration in MRS (g/L)***
Peptone	15.4	10	1.54
Meat extract	13.9	8	1.11
Yeast	11.0	4	0.44
Total MRS			3.09

APPENDIX D – Fermentation parameters

Fermentation parameter choices are based on existing literature and on general knowledge. They were not based on one paper but as an average of conditions/rates from different references. They were also considered as reasonable assumptions by different bioengineering specialists.

Direct fermentation of starch is considered as an immature process (no existing industrial-scale), so optimization over the next years is envisaged and was considered in the parameters setting, by staying conservative with literature. By conservative, it should be understood that the risk of overestimating impacts is favored than underestimating them. Fermentation parameters are therefore different from the laboratory ones. All parameters related to the bacteria were provided by publications on *L. amylophilus* from ATCC bank, and also on *L. amylophilus* GV6 strain, which is genetically more adapted to starch hydrolysis.

New technologies might have been implemented for industrial scale lactic acid fermentation but are not yet detailed in the literature. Following the conservative philosophy, the existing given information available in literature will be taken.

Fermentation of dextrose from corn

Mode: batch

- Datta et al. (2006): Mentions that industrial lactic acid facility operates as batch or fed-batch mode;
- Galactic (2009): "(…) at present, the industry still operates batch fermentation systems (…)".

Fermentation pH: 6

- Typical pH optima for lactic acid fermentation growth;
- Datta et al. (2006): Mentions that industrial fermentation pH is around 5 and 6;
- Carlon et al.(2002) & Joglekar et al.(2006): Lower pH fermentation can be realized, reducing the amount of gypsum formed. However, it is underlined in literature that low pH is still not a commercially viable preposition.

Temperature: 45°C

- Carlson et al. (2002): A high temperature (45°C-50°C) minimizes the risk of contamination;
- Eyal et al. (2001): "Typically, such production (large-scale, industrial, bacterially-conducted lactic acid fermentation process) occur at broth temperature of at least 45°C".

Yield and concentration: 90% (g lactic acid/g glucose x 100%); 92 g/L of glucose

- Datta et al. (2006): Mentions that industrial lactic acid facility has normally 90% yield;
- Cao et al. (2001) & Datta et al. (2006): The solubility of calcium lactate in water was chosen as the limiting factor for the final choice of glucose concentration since it is preferable to have calcium lactate in solution to prevent its retention during the filtration of the insoluble parts. Solubility of $_{L}$-calcium lactate in water at 45°C is about 10% (g anhydrous $_{L}$-calcium lactate/100 g of water x 100%) and was taken as an approximation of its solubility in the media. This corresponds to approximately 82.6 g/L of lactic acid and an initial glucose concentration of 92 g/L. This is in agreement with Datta et al. (2006) who mention that final calcium lactate in industrial lactic acid fermentation is around 10 wt%.

Fermentation time length: 72 hours

- Datta et al. (2006): Mentions that fermentation time in the industry is of 2-4 days.

Pretreatment of the media: none

- Bohlmann (2010): Dextrose is soluble in water and sterilization should be unnecessary, since bacteria are used. This type of fermentation is less sensitive to contamination, even more if it operates at a high fermentation temperature.

Biomass production: C.F.U. = 1.5 x 10^9 (same as laboratory)

- Arcand (2009): Laboratory biomass formation is a good approximation knowing that a cell density will not increase a lot with substrate concentration due to inhibition phenomenons.

Nutrient source and concentration: corn steep liquor (CSL), 45 g/L CSL solids

- Carlson et al. (2002): 45 g/L of corn steep liquor solids is the highest recommended concentration. The nitrogen content of this CSL concentration is practically identical to the one of MRS medium, which was evaluated during laboratory experiments (see Appendix C).

Power of agitation: P/V constant for scale-up

- Hemrajni (2007): For scale-up, the power per unit of volume will be maintained constant. This is a well employed method for scale-up of agitation. At lab scale, a speed of 50 RPM for a 2 liters fermentor was chosen, to minimize oxygen transfer in the media.

Fermentation of potato waste starch from wastewater

Mode: batch

- Same references.

Fermentation pH: 6

- Typical pH optima for lactic acid fermentation growth;
- The pH should favor the hydrolysis step, which is the limiting one, without compromising growth;
- Vishnu et al. (2000) & Vishnu et al. (2006): Optimal pH for enzymatic activity and growth *(L. amylophilus GV6*) is 6.5
- Pompeyo et al. (1993): Optimal pH for enzymatic activity *(L. amylophilus* NRRL B-4437) is between 5 and 6

Temperature: 37°C

- Typical temperature optima for lactic acid fermentation growth;
- The temperature should favor the hydrolysis step, which is the limiting one, without compromising growth;
- ATTC (2009): ATCC mentions that their cultures *(L. amylophilus* NRRL B-4437) are grown at 30ºC, but it does not mean it is the optimal one. A temperature of 37ºC should improve the hydrolysis of starch, which is the limiting step;
- Vishnu et al. (2000) & Vishnu et al. (2006): Optimal temperature for enzymatic activity and growth (*Lactobacillus amylophilus) GV6* is 37ºC;
- Pompeyo et al. (1993): Results show a decrease of amylase activity *(L. amylophilus* NRRL-4437) at a temperature higher than 40 ºC.

Fermentation time: 72 hours

- There was no publication found with *L. amylophilus* fermentation that shows a production of lactic acid at 90% yield below 72 hours. A fermentation time of 2-2.5 days could be expected following some further optimizations in the future. This parameter could have been evaluated as a variant. However, it will only influence the electricity consumption during fermentation, which has a low impact on total energy consumption. It will also have an influence on the infrastructure size, which is not part of this study. Therefore this variant was not evaluated.
- Savard (2009): Over 72 hours, it is assumed as not economically viable.

Yield and concentration: 90% (g lactic acid/g glucose); 40g/L of potato waste starch

- Datta et al. (2006): Since literature mentions that industrial lactic acid facility has normally 90% yield, this parameter will be set at this value ;
- To predict the maximal concentration of starch at which a *Lactobacillus amylophilus* strain can reach a 90% yield within 72 hours and at which viscosity is not critical for operations is impossible without advanced experiments of optimization. The viscosity and digestibility of a gelatinized starch solution is function of the starch characteristics and are influenced by numerous factors, such as the date of extraction, the type of cultivars, the method of preparation, the presence of other components, etc (see section Bibliography). The lactic acid production rate obtained is also function of the inoculum metabolic properties. From these statements, it is hard to predict the final viscosity of a specific potato starch media after heating/cooling in an optimized industrial process. It is also difficult to predict the lactic acid yield that would be obtained with an optimized inoculum of *L. amylophilus*. As underlined in the state-of-art, only a few publications exist with *L. amylophilus*. Also, literature demonstrates that, presently, the studied strains of this Lactobacilli specie cannot reach a high lactic acid rate at high concentration. To stay conservative, it has been chosen a 40 g/L potato waste starch medium would reach a

90% yield within 3 days taking into account processing, pre-culture and genetic optimization and the following references;

- Vishnu et al. (2002): Presents results showing a fermentation yield of 80% at 40 g/L of potato starch concentration with *L. amilophilus GV6* within 3 days;
- Tester et al. (2004): A general statement about starch solution mentions that a thin paste is formed when starch is heated at concentration of ~< 4*%*;

Pretreatment of the media: heating at 90°C

- Qiang (2009): In excess water (> 70 wt% water), the degree of gelatinization (based on birefringence measurements) of different potato starch cultivars studied was practically the same between 90ºC and 130ºC. Granules heated at 90°C in excess of water are also highly sensitive to shear;
- Swinkels (1985): Tuber can be gelatinized to a completely dissolved state at about 100°C;
- Tester (2004) & (T. Zhang, et al., 1999): Crystallinity is a predominant regulator in controlling susceptibility of starch to enzyme hydrolysis; therefore, a higher temperature than 90°C for heating a solution of starch in excess of water should not improve a lot the hydrolysis rate.

Biomass production: C.F.U. = 5.4 x 10^9 (same as laboratory)

- Same references.

Nutrient source and concentration: corn steep liquor (CSL), 45 g/L of CSL solids

- Same reference.

Power of agitation: P/V constant for scale-up

- Hemrajani (2007): The power per unit of volume will be maintained constant for scale-up. At lab scale, a speed of 100 RPM was chosen, due to higher viscosity of potato starch media. For scale-up, it will be supposed that it has been lowered to 50 RPM after 24h since the viscosity was similar to the one of water at that time.

APPENDIX E – Mass and energy balances

Basis of calculation

The basis of calculation is 21 590 kg of humid potato waste starch (13% humidity) fermented per week, which corresponds to 18 784 kg of dry potato waste ("PWS water" scenario). In the case of the glucose fermentation, the basis of calculation is 18 784 kg of glucose fermented per week ("Corn" scenario). Both bases of calculation lead to the same theoretical lactic acid production rate. Mass and energy flows are rounded to five significant digits.

Assumptions and statements

A list of assumptions and statements have been fixed, to realize and simplify calculations:

1. Effect on volume by carbohydrate, nutrients and biomass (dry weight) are considered as negligible. Only the total water content (including cell water content) is used for calculating the liquid volume, with $\rho_{water} = 1000$ kg/m^3;

2. Inoculum propagation is not presented in the calculations. In general, inoculum is grown by decupling the culture volume at each transfer, up to the desired inoculation volume. If we consider that the inoculum is prepared with part of the medium needed to reached the set final lactic acid concentration, only the electrical energy and cooling water consumption during decupling are not calculated;

3. Evaporation is negligible during the fermentations;

4. Even though substrates have a low percentage of impurities, they will be considered as 100% pure for simplication of yield calculations;

5. Due to the sensitivity of lactic acid to condensate during heating, the temperature in the multi-effect evaporator has to be chosen with care. The chosen conditions are: T_1 = 89°C ; T_2 = 76°C ; T_3 = 56°C, as designed by Akerberg et al. (2000);

6. Due to its low volatility compared to water (Holten, 1971), the lost of lactic acid in the multi-effect evaporator was not considered in this study, since it should be negligible;

7. At pH 6, 100% of lactic acid is under its dissociated form (calcium lactate);

8. Any buffer effect by the medium (corn steep liquor solids majorly due to its protein content) on pH is neglected (worst case philosophy);

9. Tap water is considered as neutral. Corn steep liquor has a pH of about 5.5 at 45g/L (see Appendix C), so the addition of calcium lactate for setting the initial pH is negligible. Substrates effect on initial medium pH is considered as negligible;

10. Potato waste starch hydrolysis should be expected to be the limiting step, so the concentration of glucose during potato waste starch fermentation is considered as zero;

11. During hydrolysis of potato starch, 1 kg of starch leads to 1.1 kg of glucose (Anuradha, et al., 1999);

12. The residual potato waste starch and the corn steep solids left at the end of the fermentation are considered as 100% solubilized in this study since it is difficult to predict the final solubility of these components without laboratory testing. It will be considered that these components are not filtrated by the microfiltration and the rotary filtration. It should be kept in mind that if insoluble parts are left or formed during acidification, they could be filtrated in the microfilter and the gypsum removal filter units before the evaporator;

13. Gelatinization of potato starch is an irreversible phenomenon, which happens approximatly between 58°C and 65°C for patato starch (Q. Liu, et al., 2002);

14. The heat capacity of potato waste starch is assumed to be 1.22 J/g·K (International Starch Institute, 2009) and was assumed to be constant before and after gelatinization;

15. When potato starch gelatinized, its heat capacity varies with temperature. The variation of enthalpy associated with gelatinization was considered to be 16.3 kJ/kg (Q. Liu, et al., 2002);

16. Biomass and gypsum in the retentate each have a content of 20 wt % of water, excluding cell water content:

$$\frac{m_{water}}{m_{water} + m_{biomass/gypsum}} \times 100\% = 20\%$$

Solubilized components (substrate, corn steep liquor solids and lactic acid) will be lost with the water staying in retentate. A water content of 20 wt% is typical of filtration retentate (Arcand, 2009);

17. The efficiency of removing biomass and gypsum by the filtration units is assumed to be 100%;

18. No excess of sulfuric acid for acidification was considered in this study;

19. The final biomass will be approximated following these equations and assumption 1:

$$C.F.U. = A \times 10^9$$

$$A \times 1.04[=]\, kg_{dry\ biomass}/m^3 \text{ (Timbuntam, et al., 2006)}$$

$$A \times 1.04 \times 5.71 \left(\frac{kg_{wet\ biomass}}{kg_{dry\ biomass}}\right)[=]\, {kg_{wet\ biomass}}/{m^3} \text{(Santivarangkna, et al., 2006)}$$

$$A \times 1.04 \times 5.71 \left(\frac{kg_{wet\ biomass}}{kg_{dry\ biomass}}\right) \times \frac{m_{water,\, t_0}(kg)}{\rho_{water}(\frac{kg}{m^3})}\ [=] kg_{wet\ biomass}$$

The water taken for biomass formation will be considered;

20. The agitation motor energy efficiency is 0.92 (Kampen, 1997);

21. The temperature delta of cooling water in the heat exchanger is -5°C (worts case philosophy);

22. Tap water taken for the fermentor medium is at 15°C;

23. Saturated steam at 121°C, 1.1 bar is the heating agent;

24. There is no condensate recuperation for heat transfer;

25. The specific molar heat capacity of calcium lactate can be approximated with:

$$Cp_{calcium\ lactate} = \frac{M_{lactate}}{M_{calcium\ lactate}} \times Cp_{lactic\ acid(l)} + \frac{M_{Ca}}{M_{calcium\ lactate}} Cp_{Ca(s)}$$

$$[=]\ J/g \cdot K$$

26. The specific heat capacity of a solution can be approximated using the weighted average of the constituent specific heat capacities, based on the approximation method proposed by Dimoplon (1972):

$$Cp_{sln} = \sum xi \times Cp_i [=]\ J/mol \cdot K$$

27. The reactions heats are approximated with the enthalpy of reaction. The standard heat of reaction is evaluated with the enthalpy of formation (std cond.: 298K, 1 atm):

$$\Delta H_{reaction} = \sum v_{i_{products}} \times \Delta H^o_{fi}{}_{products}$$

$$- \sum v_{i_{reactants}} \times \Delta H^o_{fi}{}_{reactants} [=]\ J/mol$$

28. The standard heat of formation ($\Delta H_f°$) of biomass is negligible in the heat reaction of lactic acid synthesis from glucose since the dry mass concentration of biomass is negligible compared to other components (from reading Von Stockar (1993)). This method of approximation was also taken by Cable et al. (1971);

29. When data are not available:

$$\Delta H^o_f(s) \approx \Delta H^o_f(aq)$$

$$Cp(s\ or\ l) \approx Cp(aq)$$

30. Heat of reaction associated with starch hydrolysis was not considered in the heat balance, due to a lack of valuable data in the literature;

31. Corn steep liquor solids and biomass were not considered in heat balances, due to a lack of thermodynamic properties in the literature;

32. The residual corn steep liquor solids at the end of the fermentation was approximated through a global mass balance. To do so, the quantity of water produced during fermentation reactions was considered to respect the mass balance equilibrium;

33. Corn steep liquor may contain traces of sugars and lactic acid, which vary from one producer to another. Sugar and lactic acid content of corn steep liquor was considered as zero in this study. It is be assumed as having 50 wt% solids and 25 wt% of protein (Davis, 2001);

34. The initial viscosity of the potato waste starch medium will be set at the approximated viscosity of the homogenous one produced in laboratory, which is more or less 35E-03 Pa·S. This is considered as the worst case scenario;

35. Agitations during gelatinization/acification were not considered, since they are performed during a short time period. Energy consumption per kg of lactic acid is supposed to be low compared to other energy consumptions;

36. The process is insulated with the environment;

37. In the multi-effect evaporator, the boiling-point elevation ΔT of lactic acid concentrate solution is small,

$$\Delta T = 5.32 \cdot x_{lactic\ acid} = 5.32 \cdot 0.88 = 4.7$$ (Akerberg, et al., 2000)

and the solution is fed at the boiling point of the effect, so

$$q_1 = q_2 = q_3 = q_i [=]\ W$$ (Geankopolis, 1993)

Assuming that the product of the global heat transfert coefficient (U) with the aire of transfert (A) is constant in each effect, therefore (see Appendix F):

$$q_i = q_{1\ effet}/3 [=]\ W$$

Steam requirement for the evaporator will be approximated following this equation:

$$\frac{\left(m_{total\ water\ to\ evaporate} \times \Delta H_{v,1\ effect}\right)}{3} = m_{saturated\ vapor} \times \Delta H_{v\ ,saturated\ steam}$$

$$\Delta H_{v,1\ effect} = average\left(\Delta H_{v,89°C}; \Delta H_{v,76°C}; \Delta H_{v,56°C}\right)$$

The quantity of cooling water needed to condensate the vapor at the last effect will be approximated as shown below:

$$\frac{\left(m_{total\ water\ to\ evaporate} \times \Delta H_{v,effect\ 3}\right)}{3} = m_{cooling\ water} \times Cp_{water} \times \Delta T_{cooling\ water}$$

$$\Delta H_{v,effect\ 3} = \Delta H_{v,56°C}$$

Fermentations planning

Assumptions:

- 1 week end off /2 weeks
- Emptying/filling should take 3h maximum each (Arcand, 2009)
- Gelatinization should not take no more than 1h
- 1 fermentor available (a second might be available but only as a back up)

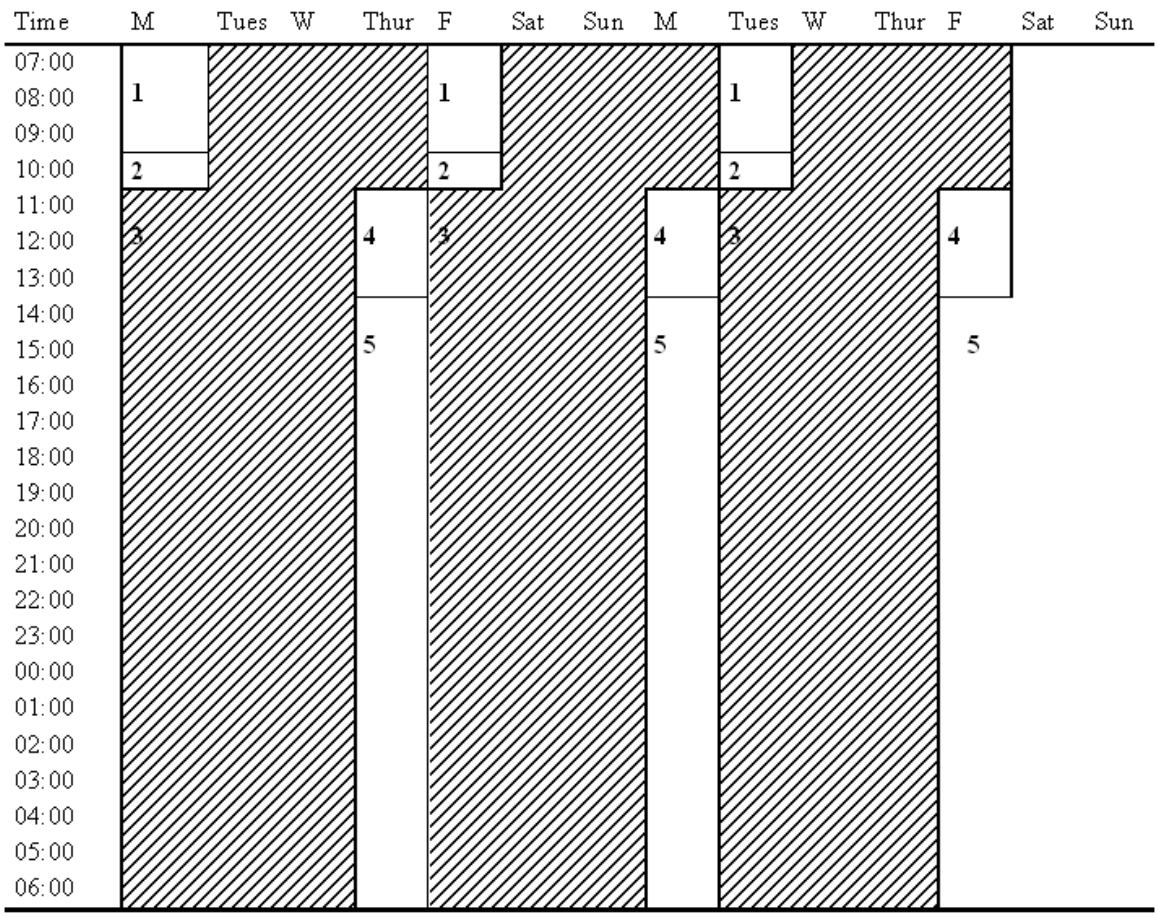


Figure E-1: Planning of glucose and potato waste starch fermentations (1.5 batches/week)
1: filling; 2: gelatinization; 3: fermentation; 4: emptying; 5: cleaning/off

Thermodynamic properties

Table E-1: Molecular weight and standard heat of formation

Component	***Molecular formula***	***Molecular weight (g/mol)***	***Heat of formation, $\Delta H_f°$ std (25°C, 1 atm) (kJ/mol)***	***Reference***
Potato Starch (s)	$(C_6H_{10}O_5)_n$	N/A	N/A	
Glucose (aq)	$C_6H_{12}O_6$	180	-1262.4	[1]
L-Lactic acid (aq)	$C_3H_6O_3$	90.1	-686.30	[1]
Calcium hydroxide (s)	$Ca(OH)_2$	74.1	-986.09	[2]
Calcium (s)	Ca	40.1	0	
Calcium lactate (aq)	$C_3H_5O_3^-Ca^+O_3^-H_5C_3$	218	-1686.1	[3]
Sulfuric acid (aq)	H_2SO_4	98.1	-907.51	[4]
Gypsum (s)	$CaSO_4$	136	-1434.5	[2]
Water (l)	H_2O	18.0	-285.84	[4]
Biomass (s)	$CH_{1.62}O_{0.38}N_{0.23}$	22.9	N/A	[1]

References: [1]: Von Stockar et al. (1993) ; [2]: Yaws (2009) ; [3]: Cable et al. (1971); [4]: Felder et al. (2000)

Table E-2: Molar heat capacities

	Heat capacities (J/mol·K) $C_p = A + B \times T + C \times T^2 + D \times T^3 + E \times T^4 + F \times T^5 + G \times T^6$							*Ref.*
Component	***A***	***B***	***C***	***D***	***E***	***F***	***G***	
Potato Starch (s)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Glucose (aq)	305.400	0	0	0	0	0	0	[1]
Lactic acid (l)	99.362	0.75655	-0.0019960	2.3739E-06	0	0	0	[2]
Calcium hydroxide (s)	-29.092	0.78856	-0.0019676	2.6339E-06	1.8948E-09	6.8177E-13	-9.4767E-17	[2]
Calcium (s)	14.162	0.084078	-0.00028173	6.4853E-07	-8.7917E-10	6.1049E-13	-1.6383E-16	[2]
Calcium lactate (aq)	210.99	1.5833	-0.0042359	5.3444E-06	-8.6031E-10	5.9740E-13	-1.6032E-16	
Sulfuric acid (l)	26.004	0.70337	-0.0013856	1.0342E-06	0	0	0	[2]
Gypsum (s)	70.207	0.098743	0	0	0	0	0	[2]
Water (l)	75.400	0	0	0	0	0	0	[3]
Biomass (s)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	

References: [1]: Von Stockar (1993); [2]: Yaws (2009); [3]: Felder et al. (2000)

Table E-3: Specific heat capacities

	Heat capacities (J/g·K) $C_p = A + B \times T + C \times T^2 + D \times T^3 + E \times T^4 + F \times T^5 + G \times T^6$							*Ref.*
Component	*A*	*B*	*C*	*D*	*E*	*F*	*G*	
Potato Starch (s)	1.22	0	0	0	0	0	0	[1]
Glucose (aq)	1.6967	0	0	0	0	0	0	[2]
Lactic acid (l)	1.1028	0.0083968	-2.2154E-05	2.6347E-08	0	0	0	[3]
Calcium hydroxide (s)	-29.092	0.78856	-0.0019676	2.6339E-06	1.8948E-09	6.8177E-13	-9.4767E-17	[3]
Calcium (s)	14.162	0.084078	-0.00028173	6.4853E-07	-8.7917E-10	6.1049E-13	-1.6383E-16	[3]
Calcium lactate (aq)	210.99	1.5833	-0.0042359	5.3444E-06	-8.6031E-10	5.9740E-13	-1.6032E-16	
Sulfuric acid (aq)	26.004	0.70337	-0.0013856	1.0342E-06	0	0	0	[3]
Gypsum (s)	0.51623	0.00072605	0	0	0	0	0	[3]
Water (l)	4.18889	0	0	0	0	0	0	[4]
Biomass (s)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	

References: [1]: International Starch Institute (2009) [2]: Von Stockar et al. (1993); [3]: Yaws (2009); [4]: Felder et al. (2000)

Table E-4: Enthalpies of vaporization and gelatinization

Type	*Component*	*Temperature(s) (°C)*	*Enthalpy (kJ/kg)*	*Reference*
Vaporization	Water	121	2139.1	[1]
		89	2222.5	
		76	2254.6	
		56	2302.1	
Gelatinization	Potato starch	58-65	16.3	[2]

References: [1]: Felder et al. (2000); [2]: Liu et al. (2002)

Needed data for mass and energy balance calculations

Table E-5: Data related to the fermentation of dextrose

Data		***Value***	***Ref.***
Medium composition (kg)	Glucose (dry weight)	92	
	CSL solids (dry weight)	45	
	Water	1000	
Substrate concentration (kg glucose /m^3)		92	
Fermentation time (hrs)		72	
Fermentation temperature (°C)		45	
Final yield (g lactic acid/g glucose)		0.9	
Biomass production (CFU)		1.5E+09	
Dry cell concentration (dry g/L)		1.56	
Water cell content (g H2O/dry g)		4.71	[1]
Wet cell weight/dry cell weight (wet g/dry g)		5.71	[2]
Wet cell concentration (wet g/L)		8.91	
Delta T for cooling (°C)		5	
Water in filtrate retentate (% w/w)		20	
Viscosity	Water (25oC) Pa·s	0.001	
	Laboratory medium t= t_0 Pa·s	0.001	
	Laboratory medium t = t_f Pa·s	0.001	
Speed of agitation	Laboratory medium t= t_0 RPM	50	
	Laboratory medium t = t_f RPM	50	
Final lactic acid concentration (% w/w)		88	
Tap water temperature (°C)		15	
Laboratory impeller diameter (m)		0.065	
Laboratory fermentor volume (m^3)		0.002	
Motor efficiency (%)		92	[3]

References: [1]: Timbuntam et al. (2006); [2]: Santivarangkna et al. (2006); [3]: Kampen (1997)

Table E-6: Data related to the fermentation of potato waste starch

Data		***Value***	***Ref.***
Medium composition (kg)	Waste (dry weight)	40.0	
	CSL solids (dry weight)	45.0	
	Water	1000	
Substrate concentration (kg humid PWS/m^3)		46.0	
Fermentation time (hrs)		72	
Fermentation temperature (°C)		37	
Yield (g lactic acid/g dry PWS)		0.9	
Biomass production (CFU)		5.4E+09	
Dry cell concentration (dry g/L)		5.62	
Water cell content (g H2O/dry g)		4.71	[1]
Wet cell weight/dry cell weight (wet g/dry g)		5.71	[2]
Wet cell concentration (wet g/L)		32.1	
Delta T for cooling (°C)		5	
Water in filtrate retentate (% w/w)		20	
Viscosity	Water (25oC) Pa·s	0.001	
	Laboratory medium t = t_0 Pa·s	0.035	
	Laboratory medium t = t_f Pa.s	0.001	
Speed of agitation	Laboratory medium t = t_0 RPM	100	
	Laboratory medium t = t_f RPM	50	
Final lactic acid concentration (% w/w)		88	
Tap water temperature (°C)		15	
Laboratory impeller diameter (m)		0.065	
Laboratory fermentor volume (m^3)		0.002	
Motor efficiency (%)		92	[3]

References.: [1]: Timbuntam et al. (2006); [2]: Santivarangkna et al. (2006); [3]: Kampen (1997)

Reactions occurring

- During fermentation

1) *Saccharification of starch into glucose molecules:*

(potato waste starch process only)

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6$$

2) *Fermentation of glucose into lactic acid:*

$$C_6H_{12}O_6 \xrightarrow{fermentation} 2C_3H_6O_3 + biomass$$

3) *Calcium lactate formation:*

$$2C_3H_6O_3 + Ca(OH)_2 \rightarrow C_3H_5O_3^-Ca^+O_3^-H_5C_3 + 2H_2O$$

4) Global reaction 2) + 3):

$$C_6H_{12}O_6 + Ca(OH)_2 \rightarrow C_3H_5O_3^-Ca^+O_3^-H_5C_3 + 2H_2O + \text{biomass}$$

$$\Delta H_{rxt}^{o} = -9.29 \text{ kJ/mol}$$

- During acidification

$$C_3H_5O_3^-Ca^+O_3^-H_5C_3 + H_2SO_4 \rightarrow CaSO_4 + 2C_3H_6O_3$$

$$\Delta H_{rxt}^{o} = -213 \text{ kJ/mol}$$

Glucose process mass and energy balances (/batch; scenario "Corn")

Raw materials and products inputs/outputs:

Table E-7: Input/output of the fermentor of scenario "Corn"

In	***kg***	***Out***	***kg***
Glucose	12 522	Glucose	1 252.2
Water	129 990	Water	137 370
$Ca(OH)_2$	4 634.4	Calcium lactate	13 634
CSL (50 wt% solids)	12 250	Biomass (wet weight)	1 212.4
		CSL solids (dry weight)	5 929.1

Table E-8: Input/output of the microfiltration unit of scenario "Corn"

In	***kg***	***Out***		***kg***
Glucose	1 252.2	Retentate	Glucose	2.763 2
Water	137 370		Water	303.11
Calcium lactate	13 634		Calcium lactate	30.085
Biomass	1 212.4		Biomass	1 212.4
CSL solids	5 929.1		CSL solids	13.083
		Permeate	Glucose	1 249.5
			Water	137 060
			Calcium lactate	13 604
			CSL solids	5 916.0

Table E-9: Input/output of the acification unit of scenario “Corn”

In	***kg***	***Out***	***kg***
Glucose	1 249.5	Glucose	1 249.5
Water	137 060	Water	137 060
Calcium lactate	13 604	Lactic acid	11 245
Sulfuric acid	6 121.9	Gypsum	8 487.1
CSL solids	5 916.0	CSL solids	5 916.0

Table E-10: Input/output of the rotary filtration unit of scenario “Corn”

In	***kg***	***Out***		***kg***
Glucose	1 249.5	Retentate	Glucose	19.342
Water	137 060		Water	2 121.8
Lactic acid	11 245		Lactic acid	174.079
Gypsum	8 487.1		Gypsum	8 487.1
CSL solids	5 916.0		CSL solids	91.579
		Permeate	Glucose	1 230.1
			Water	134 940
			Lactic acid	11 071
			CSL solids	5 824.4

Table E-11: Input/output of the multi-effect evaporator of scenario “Corn”

In	***kg***	***Out***		***kg***
Glucose	1 230.1	Condensate	Water	1 509.7
Water	134 940		Lactic acid	11 071
Lactic acid	11 071		+ impurities	
CSL solids	5 824.4	Vapor	Water	133 430

Other inputs (saturated steam, cooling water and electricty):

Table E-12: Steam, cooling water and electricity input for the fermentor of scenario “Corn”

Step	***Input***	***Value***
Pre-heating	Saturated steam (kg)	8 294.3
Agitation	Electricity (kWh)	18.820
Heat exchanger (coil/jacket)	Cooling water (kg)	20 894

Notes to Table E-12:

- $\Delta H_{rxt\ global\ 45oC}$ = -5.91 kJ/mol (+ heat by agitation) so cooling is needed during fermentation;
- By “pre-heating”, it is understood the heating of the medium to reach the fermentation temperature set point, which is 45°C;

Table E-13: Steam and cooling water for the multi-effect evaporator of scenario “Corn”

Step	***Input***	***Value***
Pre-heating (45°C → 89°C)	Saturated steam (kg)	5 959.3
Vaporization	Saturated steam (kg)	46 986
Cooling	Cooling water (kg)	4.888 8E+06

Notes to Table E-13:

- By “pre-heating”, it is understood the additional steam required to heat the medium to the first effect temperature (89°C) before vaporization;
- For the calculation of steam requirement for the “pre-heating step”, the heat released by the acidification reaction, which is exothermic, ($\Delta H_{rxt,45°C}$ = -215 kJ/mol), was taken into account;
- The cooling step is the one necessary to condensate the steam produced by the third effect of the evaporator, through a heat exchanger;

Potato waste starch process mass and energy balances (/batch; scenario "PWS water")

Raw materials and product inputs/outputs:

Table E-14: Input/output of the fermentor of scenario "PWS water"

In	***kg***	***Out***	***kg***
PWS (13% humidity)	14 394	PWS (dry weight)	1 252.2
Water	297 103	Water	306 230
$Ca(OH)_2$	4 634.4	Calcium lactate	13 634
CSL (50 wt% solids)	28 176	Biomass (wet weight)	10 039
		CSL solids (dry weight)	13 154

Table E-15: Input/output of the microfiltration unit of scenario "PWS water"

In	***kg***	***Out***		***kg***
PWS	1 252.2	Retentate	PWS	10.263
Water	306 230		Water	2 509.8
Calcium lactate	13 634		Calcium lactate	111.74
Biomass	10 039		Biomass	10 039
CSL solids	13 154		CSL solids	107.81
		Permeate	PWS	1 242.0
			Water	303 720
			Calcium lactate	13 522
			CSL solids	13 047

Table E-16: Input/output of the acification unit of scenario “PWS water”

In	***kg***	***Out***	***kg***
PWS	1 242.0	PWS	1 242.0
Water	303 720	Water	303 720
Calcium lactate	13 522	Lactic acid	11 178
Sulfuric acid	6 085.2	Gypsum	8 436.1
CSL solids	13 047	CSL solids	13 047

Table E-17: Input/output of the rotary filtration unit of scenario “PWS water”

In	***kg***	***Out***		***kg***
PWS	1 242.0	Retentate	PWS	8.624 4
Water	303 720		Water	2 109.0
Lactic acid	11 178		Lactic acid	77.620
Gypsum	8 436.1		Gypsum	8 436.1
CSL solids	13 047		CSL solids	90.596
		Permeate	PWS	1 233.4
			Water	301 610
			Lactic acid	11 100
			CSL solids	12 956

Table E-18: Input/output of the multi-effect evaporator of scenario "PWS water"

In	***Kg***	***Out***		***kg***
PWS	1 233.4	Condensate	Water	1 513.7
Water	301 610		Lactic acid	11 100
Lactic acid	11 100		+ impurities	
CSL solids	12 956	Vapor	Water	300 090

Other inputs (saturated steam, cooling water and electricity):

Table E-19: Steam, cooling water and electricity input for the fermentor of scenario "PWS water"

Step	***Input***	***Value***
Gelatinization	Saturated steam (kg)	46 610
	Cooling water (kg)	3.357 1E+06
Gelatinization (optimized)	Saturated steam (kg)	21 802
	Cooling water (kg)	823 440
Agitation	Electricity (kWh)	137.34
Heat exchanger (coil/jacket)	Cooling water (kg)	47 454

Notes to Table E-19:

- $\Delta H_{rxt\ global\ 37oC}$ = -7.25 kJ/mol (+ heat by agitation) so cooling is needed during fermentation;

- There is a variant for gelatinization that will be studied for this scenario, called "optimized gelatinization". During fermentor feeding, the medium is gelatinized in an intermediate vessel furnished with an heat exhanger, continuously. Before entering the vessel, the medium is preheated to 55°C with the help of the outlet gelatinized medium through an external heat exhanger. The outlet is then cooled with cooling water to 37°C with another heat exchanger. This configuration lowers the required quantity of steam and cooling water and resembles to continous sterilizers;
- The preheating temperature, 55°C, which is just below the gelatinization temperature range of potato starch, has been chosen to prevent a high viscosity in the heat exchanger, since potato is known for its high viscosity peak during gelatinization. It is also known for its low-medium resistance to shear rate (Swinkels, 1985), so gelatinization in a vessel with mixing was decided as a conservative configuration, for lack of experimental data.

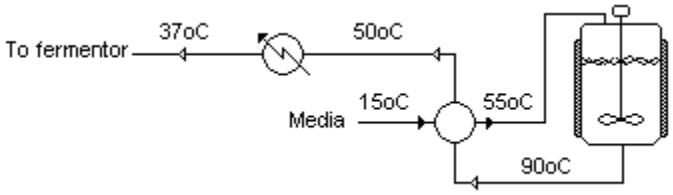


Figure 4-3: Configuration of the continuous gelatinization step

Table E-20: Steam and cooling water for the multi-effect evaporator of scenario "PWS water"

Step	***Intrant***	***Value***
Pre-heating (37° → 89°C)	Saturated steam (kg)	25 190
Vaporization 3 effects	Saturated steam (kg)	105 670
Vaporization 6 effects	Saturated steam (kg)	52 836
Cooling 3 effects	Cooling water (kg)	1.0995E+07
Cooling 6 effects	Cooling water (kg)	5.497 5E+06

Notes to Table E-20:

- By “pre-heating”, it is understood the additional steam required to heat the medium to the first effect temperature;
- For the calculation of the steam requirement for the “pre-heating step”, the heat furnished by the acidification reaction, which is exothermic ($\Delta H_{rxt,37°C}$ = -214 KJ/mol), was taken into account;
- The cooling step is the one necessary to condensate the steam produced by the third effect of the evaporator, through a heat exchanger;

Exemple of calculations

✓ Energy balances for gelatinization (not optimized; unknown variable in bold):

Heating:

$$m_{water} \times \int_{15°C}^{90°C} Cp_{water} dt + m_{potato\ waste\ starch\ (dry)} \times \int_{15°C}^{90°C} Cp_{potato\ starch} dt + m_{potato\ waste\ starch\ (dry)} \times \Delta H_{gelatinization}$$

$$= \boldsymbol{m_{saturated\ steam}} \times \Delta H_{vaporization,saturated\ steam}$$

Cooling:

$$m_{water} \times \int_{15°C}^{90°C} Cp_{water(l)} dt + m_{potato\ waste\ stahc\ (dry)} \times \int_{15°C}^{90°C} Cp_{potato\ starch(s)} dt$$

$$= \boldsymbol{m_{cooling\ water}} \times \int_{25°C}^{30°C} Cp_{water(l)} dt$$

✓ Agitation during fermentation:

$$Re = \frac{\rho \times N \times D^2}{\mu}$$

At laboratory scale:

$$Re_{t=t_0} = \frac{1052.3\frac{kg}{m^3} \times \frac{100RPM}{60s} \times (0.065m)^2}{0.02\ Pa.s} = 370 \rightarrow transional\ flow$$

$$Re_{t=t_f} = \frac{1052.3\,\frac{kg}{m^3} \times \frac{50RPM}{60s} \times (0.065m)^2}{0.001\ Pa.s} = 3705 \rightarrow transional\ flow$$

With the diagram of N_p versus Re (Hemrajani, 2007):

$$N_{p,t=t_0} = 4.7$$

$$N_{p,t=t_f} = 5$$

The power, P:

$$P = N_p \rho N^3 D^5\ [=]\ \text{W}$$

The rule of scale-up:

$$\frac{P_{laboratory}}{V_{laboratory}} = \frac{P_{industrial}}{V_{industrial}}$$

$$\frac{4.7 \times 1052.3\ kg/m^3 \times {}^{50RPM}/_{60s} \times 0.065^5}{0.002\ m^3} = \frac{P_{industrial}}{V_{industrial}}$$

✓ Heat of reactions

Enthalpy of reaction of calcium lactate precipitation to gypsum at 37°C:

$$\Delta H_{rxt,37oC} = 2 \times \Delta H^{o}_{f,lactic\ acid(l)} + 2 \times \int_{25°C}^{37°C} Cp_{lactic\ acid(l)} dt$$

$$+\Delta H^{o}_{f,gypsum(s)} + \int_{25°C}^{37°C} Cp_{gypsum(s)} dt$$

$$-\Delta H^{o}_{f,H2SO4(l)} - \int_{25°C}^{37°C} Cp_{H2SO4(l)} dt$$

$$-\Delta H^{o}_{f,calcium\ lactate(s)} - \int_{25°C}^{37°C} Cp_{calcium\ lactate} dt$$

$$= -214\ kJ/mol$$

APPENDIX F – Multi-effect evaporator theory

Detailed representation of a triple effect evaporator:

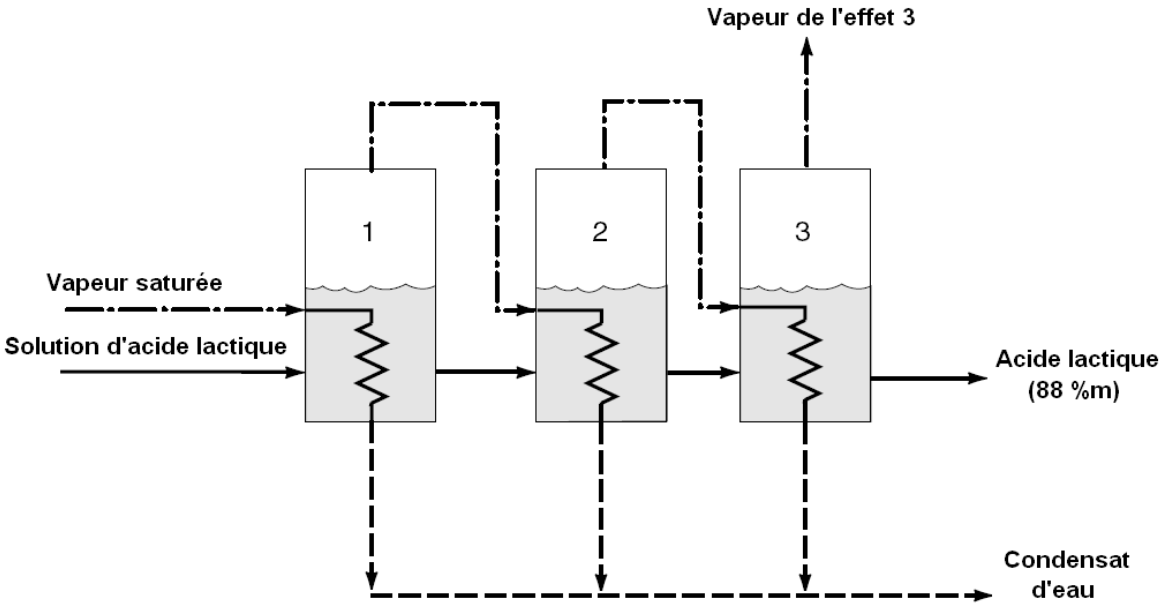


Figure F-1: Triple effect evaporator, modified from Wilf (2007)

In a multi-effect evaporator, the vapor produced in an effect is used as the heating medium for the following effect. Since the boiling temperature of the next effect has to be lower to enable heat transfer between steam and the solution, the pressure is lowered. Pressure will be reduce gradually from one effect to the other. At each effect of a three-effect evaporator:

$$q_1 = U_1 A_1 \Delta T_1$$

$$q_2 = U_2 A_2 \Delta T_2$$

$$q_3 = U_3 A_3 \Delta T_3$$

If the heat of solution and the increase of the solution boiling point at its most concentrated state are negligible, so

$$q_1 = q_2 = q_3$$

If the product of the global heat transfer coefficient (U) with the trasfer area (A) is constant in each effect,

$$U_1A_1 = U_2A_2 = U_3A_3[=]\ W/°C$$

$$q = UA(\Delta T_1 + \Delta T_2 + \Delta T_3) = UA\Delta T[=]\ W/°C$$ (Geankopolis, 1993)

So the heat transfert in each effect can be calculated from the heat transfert of a one-effect evaporator with the same UA product, divided by 3.

APPENDIX G – Allocation methods

The corn wet milling facility assumed co-product production rates per ton of dry corn transformed is presented in Table G-1:

Table G-1: Corn wet milling production yields

Substance	***P: product/ C: co-product***	***kg (dry) per ton of dry corn transformed***
Dextrose	P	720
Corn gluten feed	C	200
Corn gluten meal	C	60
Corn oil	C	20

These assumed substances production rates come from Renouf et al. (2008), from which the input/output for the production of dextrose was taken. In this study, the authors assumed a yield of 1 kg of dextrose/kg starch content (dry basis). A final purity of 96.5 wt% of dextrose (= $dextrose_{96.5}$), leading to 746 kg $dextrose_{96.5}$ /ton dry corn was considered in the study. The production rates of the different co-products per ton of $dextrose_{96.5}$ (dry basis) are shown in Table G-2:

Table G-2: Co-product yields (dry mass) per ton of dextrose produced (Renouf, et al., 2008)

Co-product	***kg (dry) per ton of dry $dextrose_{96.5}$ produced***	***Protein content (wt %)***
Corn gluten feed	268	20
Corn gluten meal	80	60
Corn oil	27	0

This dextrose yield is probably slightly overestimated, since it does not consider any starch lost in co-products. Also, this study does not consider any humidity content in co-products. However, these yields and protein content will be conserved to be coherent with the LCA inventory of Renouf et al. study. Also, corn composition is variable between cultivars and might generate differences in yields. Only one modification will be done to co-product yields to take into account a limited amount of corn steep liquor sold. As mentioned before, only some facilities sell part of their corn steep liquor production as a sole product. The processing system of Renouf et al. (2008) does not consider the selling of corn steep liquor. According to the Corn Refiners Association (2009), the US corn refining industry has sold an average ratio of 0.04kg of corn steep liquor per kg of product sold (starch, sweeteners, etc.) in 2008. Since this average is low, it should have little impact on total energy needs and on final corn gluten feed protein content. These variations will be neglected.

This ratio of 0.04 kg of corn steep liquor/kg of product produced will be used to approximate the corn steep liquor production rate per kg of $dextrose_{96.5}$. Corn steep liquor will be assumed as having a ~50 wt% solids content and ~25 wt% protein content. These values are averages from literature. The new production yields are given in Table G-3:

Table G-3: Co-products yield, taking into account the selling of corn steep liquor

Co-product	***kg (dry) per ton of dry dextrose$_{96.5}$ produced***
Corn gluten feed	268 – 20 (CSL solids) = 248
Corn steep liquor	40
Corn gluten meal	80
Corn oil	27

System expansion

The same displaced animal feed product of Renouf et al. LCA will be used. A mix of 80 kg of corn gluten meal (60% wt protein) with 27 kg of corn gluten feed (20% wt protein), which produces a 107 kg of a feed mix (~50% wt protein), was considered to be equivalent to 107 kg of soybean meal (50% wt protein).

The remaining 221 kg of corn gluten feed was considered as approximately equivalent to 221 kg of barley (10% wt protein). It is recognized as having the same Total Digestible Nutrients level as barley (Ingredients101.com). One kg of corn gluten feed will be considered as 1 kg of barley, even if protein contents are different. In the case of corn oil, it was assumed to replace the same amount of soybean oil.

It will be considered that the input of corn steep liquor used as a nutrient additive in fermentation would have to be compensated by a higher production of soybean meal. In fact, corn steep liquor is recognized as being a substitute to soybean meal, due to its high dry weight protein content (Corn Refiners Association, 2006). Since corn steep liquor has a protein content of about 25 wt%, the energy and emissions associated to 0.5 kg of soybean meal will be attributed to 1 kg of corn steep liquor, based on protein content.

A summary of the displaced products per ton of dextrose$_{96.5}$ from the co-products production rates of Table G-3 is shown in Table G-4:

Table G-4: Displaced products by the production of dextrose$_{96.5}$

Displaced product	***Quantity displaced (kg) per ton of dextrose$_{96.5}$***
Barley	221
Soybean meal	127
Soybean oil	27

Economic allocation

The different selling costs taken for calculating economic allocation percentages are presented in Table G-5:

Table G-5: Selling price of dextrose and co-products

Substance	***selling price x 10^3 (US$/kg)***	***Reference***
Dextrose	758.0	Baker et al. (2010)
Corn gluten feed	77.42	Baker et al. (2010)
Corn steep liquor	176.4	Aden et al. (2001)
Corn gluten meal	488.8	Baker et al. (2010)
Corn oil (“crude”)	722.0	Ash et al. (2010)

The price of dextrose was considered to be at 96.5% purity. The weight percentages of emissions and energy consumption ($W_{i\%}$) related to dextrose production that are allocated to dextrose and corn steep liquor were calculated following this equation:

$$w_{i\%} = \frac{Y_i \times C_i}{\sum_{i=1}^{5} Y_i \times C_i} \times 100\%$$

Where C_i is the selling cost per kg of substance and Y_i is the production rate of the substance (kg) per ton of dextrose$_{96.5}$ produced. The production rates, Y_i, are shown in Table G-13.

The allocation weight percentage is 90% for dextrose and 0.84% for corn steep liquor.