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Environmental impact Assessment of the L-lysine production process from fermentation of glucose by *Corynebacterium glutamicum* using Super Pro Designer software

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ABSTRACT/RESUME

Abstract: *The assessment key factors of any bioprocess sustainability are mainly its interaction towards the environment and the capacity to control any generated pollution, beside its social and economical aspects. These factors have direct impact on the decision-making regarding the choice of the relevant technologies at the early stages of the bioprocess design.*

Therefore, in the present study we assessed the environmental impact of a chosen model process, namely for the production L-lysine by fermentation using Corynebacterium glutamicum and glucose as the bacteria and the substrate, respectively.

We used the Super Pro Designer® version 9.0 software to compute mass balances of the entry and exit streams. The results, together with all relevant impact categories, which are classified according to calculated mass indices, were used for environmental assessment to deduce the set environmental parameters, as environmental factors and indices.

In order to optimize the environmental performance of the bioprocess, other indices were also calculated. For instance, the results showed that Water was the most influential relevant component from mass point of view with 20.97 and 22.32 kg/kg P for input and output, respectively. Regarding the fact that Water is an exhaustible natural resource, it is necessary to anticipate its recycling. Therefore, as a second step a part of the water recovered during the purification of the main product was recycled along with L-Lysine wastes. This led to important mitigations of the environmental impacts of Water and L-Lysine as well to a production increase of the latter.

I. Introduction

The pharmaceutical industry is the strategic economic sector that brings together the research, manufacturing and commercialization of drugs for human and veterinary medicine. It is one of the most profitable and economically important industries in the world. However, one of most challenges in this industry is the non-renewable aspect of raw materials leading to important environmental questions.

L-Lysine is one of the nine essential amino acids with a wide range of applications in various industries such as food, pharmaceutical and medical [1-3]. L-lysine is provided by the different companies either as a crystalline preparation containing 98.5 % L-lysine hydrochloride, as an alkaline solution of 50.7% L-lysine concentration, or as a lysine sulfate preparation containing 54.6 % L-lysine [4, 5].It can be produced either via a chemical process or a biochemical which is relatively more economic and not generate residues with a high pollutant load. L-lysine demand is significantly increasing over the years. Estimated annual lysine demand exceeds two million tons and countries like China and North America have experienced a very measured market development [4, 6 and 7]. Global Lysine market size will increase to 3910 Million US$ by 2025, from 3070 Million US$ in 2017 [8]

In addition to the stereospecificity of amino acids (the L isomer), the steadily increasing L-lysine demand strongly favors its fermentative production over chemical processing. This growth stimulated intensive research into the pathways of its synthesis as well as the search for microorganisms capable of overproducing this amino acid with the most economical synthesis method. Among the different processes, microbial fermentation is the most efficient and environmentally friendly [8]. Thus, L-lysine producing strains of the gram positive *Corynebacteria*, especially *Corynebacterium Glutamicum*, *Brevibacterium Flavum* and *Brevibacterium Lactofermentum*, have been used for the last fifty years for the industrial production of amino acids [9].

Despite the many advantages of amino acids production by fermentation, particularly L-lysine, the process still requires significant improvements leading to increased productivity, reduction of production costs and the environmental impacts, hence to a sustainable development [10]. Indeed, economical, environmental assessments and societal development are very important in assessing the sustainability of the process.

The environmental assessment includes studying the environmental impact of used resources and wastes resulting from the manufacture. This could lead to proposing solutions like recycling water to reduce freshwater consumption, since the L-lysine production process is a water consumer by excellence and may lead to environmental impacts with serious consequences on human health, ecosystem quality and the available resources. These impact categories have recently raised scientists' interest and were considered in few studies only [11, 12]. Methodological developments considering these impacts are still ongoing as illustrated by the literature review of Kounina et al. [13]. Other solutions could be proposed like the discharges treatment before disposing them off in the nature or reusing them, as is the case of the biomass produced during the synthesis, leading to a biogas production with a reuse of the remaining biomass as fertilizer.

The present work focused on the environmental assessment of L-lysine production from sugar and *Corynebacterium Glutamicum*. The ultimate goal was to reduce the raw materials consumption and increase the mean product by using recycling. The adopted method allows a global response by taking into consideration a large number of environmental impacts such as acidification, eutrophication, depletion of resources, etc. Several scenarios were considered based on the used raw materials.

On the basis of the flowsheet developed by T. Lopez-Arenas et al. [14], we assessed the environmental impacts of various process parameters in order to optimize and develop solutions that can contribute to environment protection.

II. Materials and methods

II.1. Description of the process

Generally in bioprocesses, typically complex raw materials are used as reactants or substrates for the bioreaction beside additional materials like solvents and mineral salts which are consumed in the fermentation and product separation/purification steps. Also the process requires consumables like chromatography resins and membranes, utilities like electricity, steam, cooling water, and finally workforce to run the process which for convenience can be divided into three sections: upstream, bioreaction and downstream, as shown in Figure 1.

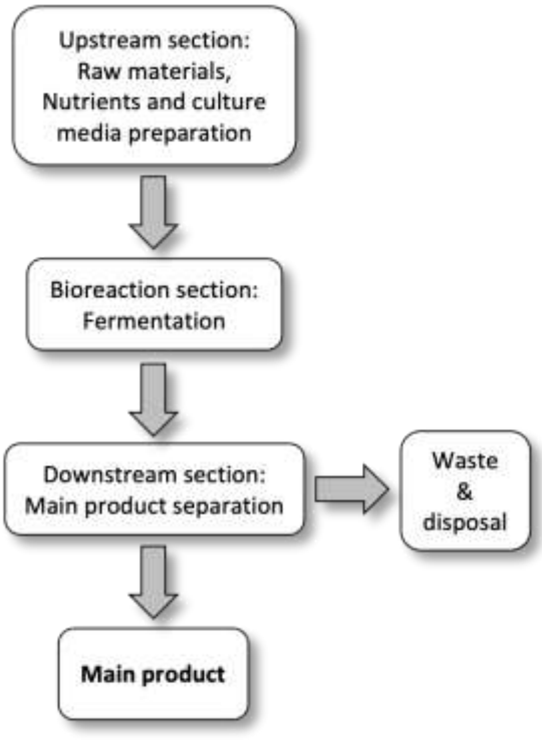

Figure 1. *The process flow diagram.*

The upstream processing includes the seed train to provide the necessary amount of inoculum and the preparation of the media for the bioreaction. The bioreaction section includes a bioreactor and all related equipments such as the compressor and air filter, before introducing it to a fermenter. The bioreaction is the central part of the process that biologically converts the raw materials into the desired product. Usually, by-products are formed and raw materials are not completely consumed, generating waste in the process. The following downstream processing section includes all steps necessary to separate and purify the product from the other materials to provide a sufficiently pure final product.

II.2. Process design, modeling and simulation

Process design is the conceptual work done prior to building, expanding or retrofitting a process plant. Mainly it consists of the process synthesis and then its analysis. The first step *i.e.* the process synthesis deals with the selected relevant set of unit operations and their arrangement in order to produce the target product with the required quality at a reasonable cost. The second step is concerned by the analysis of the process synthesis, assessing and comparing different synthesis routes. Generally a synthesis step is always followed by its analysis. The results are determinant for the subsequent synthesis steps concerning, for instance, the bioprocess design and the economics.

Process modeling is a mathematical description of a process making easy its understanding and its study under different operating conditions. Then the model can be implemented in a simulator used to develop simulations of the process operations in order to identify potential improvements as well as possible difficulties, through computers experiments which can be very useful bases for decision- making.

In the present work, a performing simulator, namely Super Pro Designer® version 9.0 has been used and has proven its great reliability in the development and analysis of various processes of different nature (industrial, environmental, etc.).

Super Pro Designer® is a simulation software, which facilitates modeling, evaluation and the optimization of integrated processes in a wide range of industries (Pharmaceutical, Biotech, Specialty chemicals, Food, Consumer goods, Mineral treatment, Microelectronics, Water purification, Wastewater treatment, Air pollution control, etc.). Super Pro Designer® enables a modeling of the manufacturing and end-of-line treatment processes, of the economic evaluation of the projects and of the environmental impact assessment.

II.2.1. Process flowsheet elaboration

The process model was built using Super Pro Designer® version 9.0 and the corresponding flowsheet as elaborated by means of this software is as shown in details in Figure 2. As mentioned above the objective of the proposed strategy is to get information concerning the eventual environmental impacts of a bioprocess. Referring to Figure 2 below, there are three main sections.

1) Culture medium preparation section

In this part of the process, water is added to substrate in the blending (P-1). On the other hand, the required amounts of nutrients and water are mixed in the blending tank (P-3). It should be noted that for the lysine yield (mol C/mol C) on glucose as substrate is 8% higher than on sucrose and 30% higher than on fructose as reported by Kiefer et al. [15]. Ammonium hydroxide (NH_4OH) and monopotassioum phosphate (KH_2PO_4), chosen as the supplier sources of nitrogen and phosphorus, respectively, threonine ($C_4H_9NO_3$), as mutant. Each mixture is then pasteurized through heat sterilizers (P-2) and (P-4) respectively.

2) Bioreaction section or fermentation section

The culture medium goes into the fermentation unit (P-11), where the biomass ($CH_{1,9}O_{0,3}N_{0,24}P_{0,02}K_{0,01}$) is added. The fermentation is carried out in the fermenter (FR-101) under aerobic conditions at a constant temperature of 35 ° C and a pressure of 1.013 bar. Since the reaction is exothermic and in order to keep the temperature constant, a jacket with cooling water is used. The ambient air is supplied by the gas compressor (G-101) to pass through the air filter (AF-101) where it is filtered before going into the fermenter. The production process of L-lysine from glucose has been previously studied by Heinzle et al. [16], where simulations were performed using an appropriate stoichiometric reaction based on the product yield and productivity. The same reaction was used by T. Lopez-Arenas et al. [14]. The biological reaction is as follows :

Fermentation:

$$C_6H_{12}O_6 + 0.022\ KH_2PO_4 + 0.985\ NH_4OH + 0.007\ C_4H_9NO_3 + 2.193\ O_2 \rightarrow 1.080\ CH_{1,9}O_{0,3}N_{0,24}P_{0,02}K_{0,01} + 0.367\ C_6H_{14}N_2O_2 + 4.925\ H_2O + 2.749\ CO_2 + 0.011\ K$$

Once fermentation is complete, the generated gases (carbon dioxide, nitrogen and oxygen) are emitted by the current (S-108) then filtered in the filter (AF-102) before being released into the environment. At the same time the broth is discharged into the reservoir (P-9), acting as a buffer reservoir, and then it goes to purification section.

3) Purification section

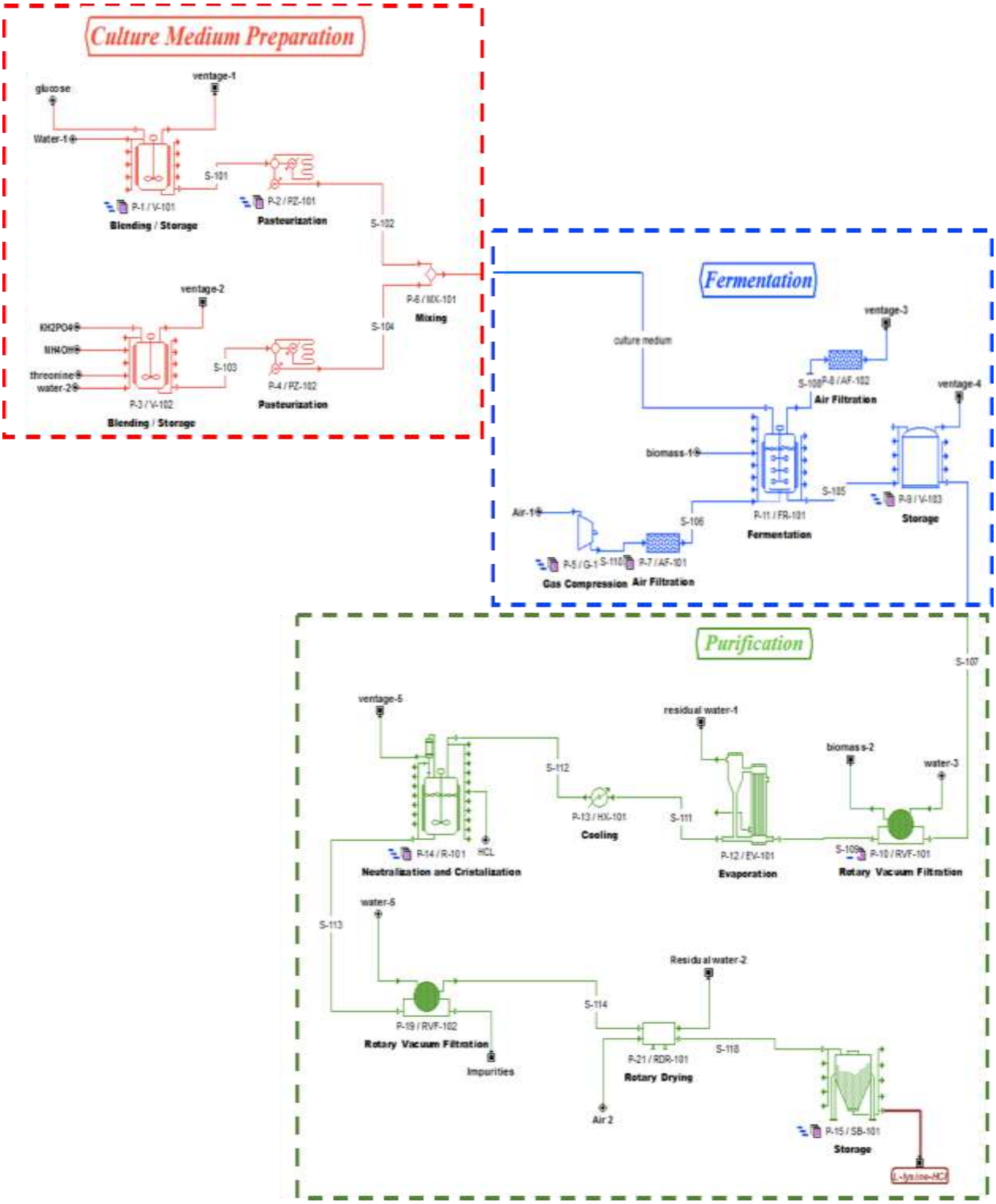

Figure 2. Process of lysine industrial production

In this downstream section, the reaction products pass into a rotary vacuum filter (P-10) to separate the biomass from the liquid. The permeate is then transferred to the evaporator (P-12) to reduce the amount of water from the stream (S-109). After cooling the mixture goes into a cristalliser (P-14). A sufficient amount of hydrochloric acid (HCl) is added for the crystallization and the neutralization of Lysine and ammonium hydroxide, respectively, at 15°C, according to the following stoichiometric reactions:

Cristallisation: 1(HCl)+1 (lysine, en solution) → 1(lysine HCl, cristaux)

Neutralization: 1 (HCl) +1 (NH_4OH) →1 (lysine HCl)

A second filtration by the rotary vacuum filter (P-19) is needed to remove impurities from the L-lysine-HCl mixture. Then the L-lysine-HCl is dried in a rotary dryer (P-21) and the residual water is removed. The L-lysine-HCl crystals are transfered to the storage tank (P-15) for further operations.

II.2.2. Environmental assessment method

The environmental method adopted in the present work was proposed by Heinzle et al. [17, 18] and used by both Nica et al. [19] and Biwer et al. [20]. It is mainly based on the calculation of environmental indices and its different steps can be illustrated by the following general environmental assessment flow diagram:

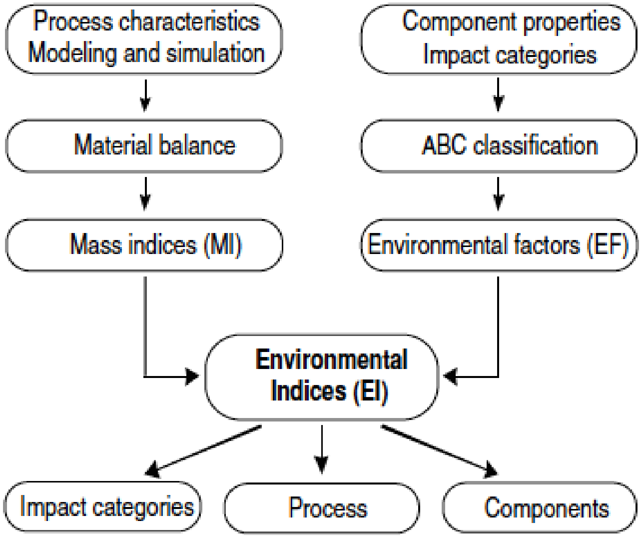

Figure 3. *Assessment the method algorithm* [20]

The method is a priori concerned by two main aspects: the process characteristics and the impacts due to the involved component properties.

II.2.2.a Indices calculation

This first aspect concerns the process and its characteristics. The corresponding indices can be obtained by means of a Super Pro Designer® simulation which is based on the calculation of the overall material balance. The latter is needed to evaluate the so-called mass Index (*MI*) for input and output components [16]. An MI indicates the amount of material consumed to produce a unit mass of the end product and is also used to calculate other important indices shown in Table 1 just below. A more detailed calculation method is given by Heinzle et al. [16, 18].

Table 1. *Calculation of weighting indices and factors.*

Weighting Factors/Indices	Definition	Calculation
Mass Index component i MI_i (kg/kg P)	INPUT: The amount of component i (mi) to produce 1kg of product (m_P). OUTPUT: The amount of component i formed per unit final product	$MI,in = \frac{mi,in}{mp}$ $MI,out = \frac{mi,out}{mp}$
Mass Index Process $MI_{Process}$ (kg/kgP)	Gives a metric for the material intensity of the process for input and output component	$MI_{Process,in} = \sum_1^i \frac{mi,in}{mp}$ $MI_{Process,out} = \sum_1^i \frac{mi,out}{mp}$
Environmental Factor component i, EF	The potential environmental relevance of a component i, derived from Impact groups for input and output component	-Via arithmetic average: $EF_{Mv,i} = \frac{\sum_1^j IGi,j}{i}$ -Via multiplication: $EF_{Mult,I} = \prod_1^j IGi,j$
Environmental Index component i, EI (Index Point/kgP)	The potential environmental relevance of a component i to produce 1kg of final product. For input and output component	$EI_i = EF_i.MI_i$ As $EI_{i,Mv}$ or $EI_{i,Mult}$
Environmental Index Process, $EI_{Process}$ (Index Point/kgP)	The overall environmental relevance of the process. For inputs and outputs	$EI_{,process} = \sum_1^i EIi$
General Effect Index process, GEI		$GEI = \frac{EIprocess}{MIprocess}$
Impact Category Index, ICI_j	The environmental impact of the component i in the category j $IC_{j,i}$	$ICI_j = \frac{\sum_1^i ICj,i.MIi}{MIprocess}$
Impact group Index, IGI_j	The environmental impact of the component i in the impact group j	$IGI_j = \frac{\sum_1^i IGj,i.MIi}{MIprocess}$

II.2.2.b. Component properties impact categories

Clearly not all components in the system have the same environmental relevance, hence the second part of the adopted approach is concerned by the need to consider impact categories in the environmental factors calculation. The classical procedure is to use Heinzle ABC classification [16, 17]. Figure 4 represents 14 impact categories and their negative effects on the environment, human health, air, water and soil.

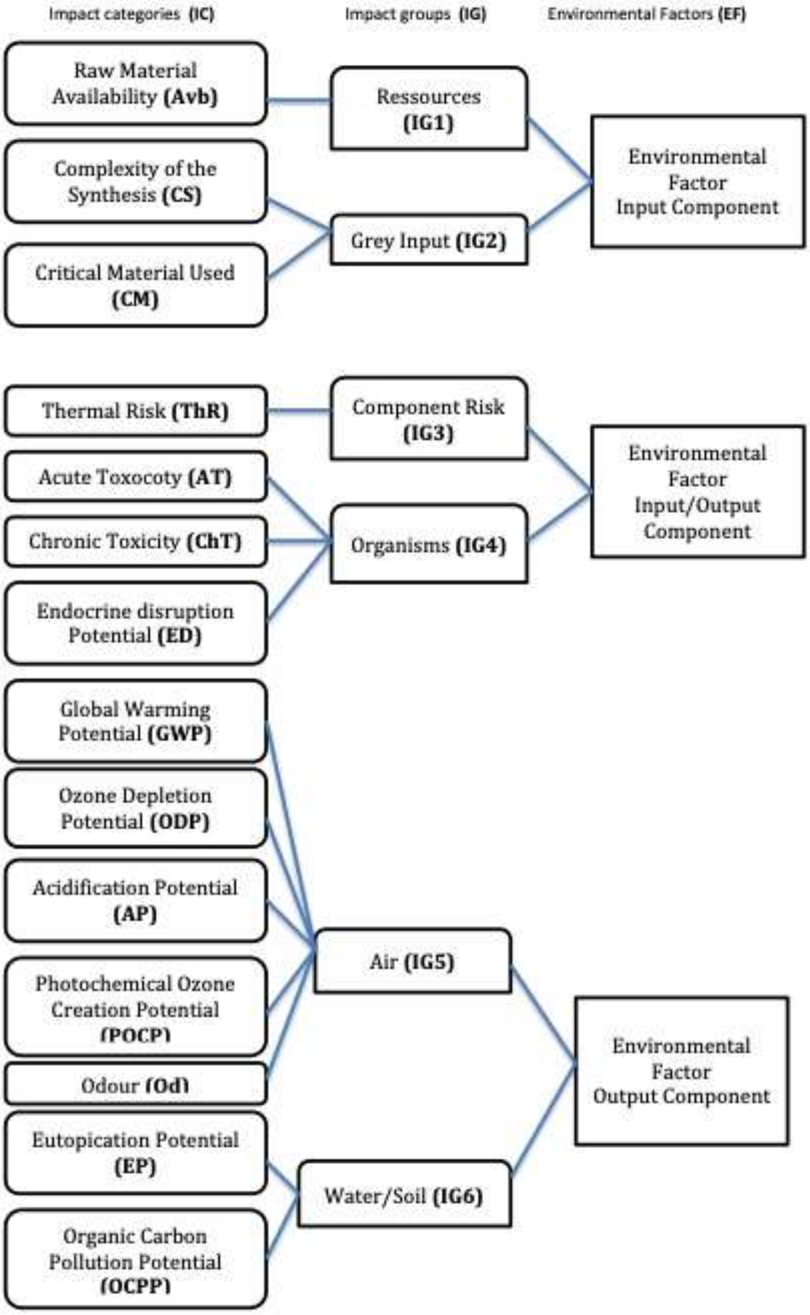

Figure 4. *Impact categories used, their allocation to the impact groups and the derivation of the Environmental Factors for input and output components. Part of this figure is taken from bibliography [17].*

II.2.2.c. Classification method

As shown in Figure 4, the EF of the inputs considers Resources, Grey Input, Component Risk and Organism impact groups, while the EF of outputs comprises the groups Air, Water/Soil, Organisms and Component Risk. In the impact groups, a component is also allocated to one of the three classes A, B, C (A for high, B for medium and C for low relevance). The highest classification in the referred ICs defines the class of the IG.

The classification is based on international classifications like R-codes, the EU hazard symbols, the flammability and reactivity hazard classes of the US National Fire Protection Agency (NFPA) that consider flammability, thermal stability, reactivity, and incompatibility with air, water, and other compounds. These classifications are available for almost every compound. The IC considers input and output components [14] but do not consider the intermediate products.

The calculation of the *EF* is determined by two factors: the numerical values of the classes and the way they are aggregated to one value. In the method presented, two options are offered by Heinzle. The EFMult uses the values *A* = 4, *B* = 1.3, and *C* = 1 and then these values are aggregated by multiplication. The alternative EFMv uses the values *A* = 1, *B* = 0.3, and *C* = 0. In this work the Material Safety Data Sheets, NFPA (National Fire Protection Association) and HMIS (Hazardous Materials Identification System) classifications for every component are used as well as Biwer et al. estimation method in some cases [19].

III. Results

In order to assess Super Pro Design® results, data reported in the literature concerning the modeling, simulation and dynamic analysis of the L-lysine production process [14] were used for this purpose. It should be stated that most of the design examples considering L-Lysine-HCl production adopt the same production flowsheet with the same basic reactions, kinetics, operating and initial conditions. However, the difference can arise at the feed raw materials level which can be a priori molasses or directly glucose as is the case of the present study.

Table 2. *Production rate and overall product Yield.*

Mode batch	From litterature [14]		From this work	
Initial threonine concentration (g/l)	Production rate (kg/batch)	Overall product Yield (%)	Production rate (kg/batch)	Overall product Yield (%)
1	21940	31	21872	30.5
1.6	16097	21.8	15805	22.1

The obtained results concerning the production rate and the production yield are very close to the values reported in the literature [14] with an absolute relative deviation of 0.3%, whereas the process times including the reaction time as well all the dead times (filling, emptying and washing) were quite different reaching 53 and 100 hours for the considered case and for the example reported in the literature, respectively. There is no evident explanation to the difference which may be due to the different composition of the considered initial feeds which were glucose and molasses, respectively, with the latter requiring several operations for their preparation, so more time.

The evaluation process resulted in the following classifications shown in Tables 3 and 4. For instance regarding the inputs, KH_2PO_4 needs a heat source and natural phosphate to be produced. This source is non-renewable as confirmed by the Global Phosphorus Research Initiative which predicts that the world could run out of Phosphorus in 50 to 100 years unless new reserves of this element are discovered [21]. Also, NH_4OH is produced using several steps. These components are classified B in resources impact group (IG1).

For the Hazards identifications, KH_2PO_4 is known to be hazardous in case of skin and eye contacts, of ingestion, and of inhalation. It is not classified according to the EU regulations but according to HMIS and NFPA as it may cause Irritation or minor reversible injury possible, it is classified 1 [22] and according to Heinzle et al table it is in class C [17].

The most hazardous for organism group with an acute toxicity are NH_4OH as nitrogen source and HCl as neutralizing/crystallizing agent. Both of them are corrosive, irritant and permeator in skin and eye contact and very hazardous in case of ingestion, so they are classified A [23, 24]. To handle these substances in the process carefully, the use of adequate protective equipment, can minimize the risk. The other components have moderate or low impact [25-30].

Solid potassium has a high relevance in thermal risk group since it may produce flammable gases in contact of water. It may also ignite spontaneouslyin contact of water or moist air. It is highly flammable in presence of open flames and sparks of heat, flammable in presence of moisture [26]. However, for the present case it is not dangerous for any group because it is formed as by-product leaving the process in soluble form with aqueous impurities. KH_2PO_4, NH_4Cl and NH_4OH as outputs are classified A due to their importance in eutrophication impact category [20, 21 and 23], but fortunately they leave the process in much reduced amounts. It should be mentioned that material classification has been carried out basing on the literature [15, 26-30]. The results of the environmental assessment are shown in the following table 5:

Table 3. *ABC- classification of the Impact categories and Impact groups of the input involved in $_{L}$-lysine-HCl production (For abbreviations of impact categories and groups see Figure 4).*

Component	Avb	**IG1**	CS	CM	**IG2**	ThR	**IG3**	AT	ChT	ED	**IG4**
Biomass	C	**C**	C	C	**C**	C	**C**	C	C	C	**C**
Gas carbon.	-	**-**	-	-	**-**	-	**-**	-	-	-	**-**
Glucose	C	**C**	C	C	**C**	C	**C**	C	C	C	**C**
KH_2PO_4	B	**B**	C	C	**C**	C	**C**	C	C	C	**C**
Lysine	-	**-**	-	-	**-**	-	**-**	-	-	-	**-**
Lysine-HCl	-	**-**	-	-	**-**	-	**-**	-	-	-	**-**
NH_4Cl	-	**-**	-	-	**-**	-	**-**	-	-	-	**-**
NH_4OH	B	**B**	C	C	**C**	C	**C**	A	B	C	**A**
HCl	C	**C**	C	C	**C**	C	**C**	A	B	C	**A**
Potassium	-	**-**	-	-	**-**	-	**-**	-	-	-	**-**
Thréonine	C	**C**	C	C	**C**	C	**C**	B	C	C	**B**
Water	C	**C**	C	C	**C**	C	**C**	C	C	C	**C**

Table 4. *ABC- Classification of the Impact categories and Impact groups of the output involved in $_{L}$-lysine-HCl production.*

Component	ThR	**IG3**	AT	ChT	ED	**IG4**	GWP	ODP	AP	POCP	Od	**IG5**	EP	OCPP	**IG6**
Biomass	C	**C**	C	C	C	**C**	C	C	C	C	C	**C**	B	B	**B**
Gascarbon.	C	**C**	C	C	C	**C**	B	C	C	C	C	**B**	C	C	**C**
Glucose	C	**C**	C	C	C	**C**	C	C	C	C	C	**C**	C	B	**B**
KH_2PO_4	C	**C**	C	C	C	**C**	C	C	B	C	C	**B**	A	C	**A**
Lysine	C	**C**	C	C	C	**C**	C	C	C	C	C	**C**	B	C	**B**
Lysine-HCl	C	**C**	C	C	C	**C**	C	C	C	C	C	**C**	B	C	**B**
NH_4Cl	C	**C**	B	B	C	**B**	C	C	C	C	C	**C**	A	C	**A**
NH_4OH	C	**C**	A	B	C	**A**	C	C	C	C	C	**C**	A	C	**A**
HCl	-	**-**	-	-	-	**-**	-	-	-	-	-	**-**	-	-	**-**
Potassium	C	**C**	C	C	C	**C**	C	C	C	C	C	**C**	C	C	**C**
Thréonine	C	**C**	B	C	C	**B**	C	C	C	C	C	**C**	B	C	**B**
Water	C	**C**	C	C	C	**C**	C	C	C	C	C	**C**	C	C	**C**

Table 5. *Environmental assessment factors and indices of $_{L}$-lysine-HCl production.*

Component	**Input**					**Output**				
	MI	EFM_V	EIM_V	EFMult	EIMult	MI	EFM_V	EIM_V	EFMult	EIMult
Biomass	0.047	0	0	1	0.047	0.43	0.3	0.13	1.3	0.55
Gascarbon	-	-	-	-	-	1.84	0.3	0.55	1.3	2.39
Glucose	2.74	0	0	1	2.74	4.57E-07	0.3	1.37E-07	1.3	5.94E-07
KH_2PO_4	0.046	0.3	0.0137	1.3	0.059	3.98E-05	0.65	2.58E-05	5.2	0.00026
Lysine	-	-	-	-	-	0.0166	0.3	0.005	1.69	0.028

Lysine-HCl	-	-	-	-	-	1	0.3	0.3	1.69	1.69
NH_4Cl	-	-	-	-	-	0.0017	0.65	0.001	5.2	0.009
NH_4OH	0.53	0.65	0.342	5.2	2.74	2.33E-05	1	2.33E-05	16	0.0004
HCl	0.201	1	0.201	4	0.803	0	-	-	-	-
Potassium	-	-	-	-	-	0.0065	0	0	1	0.0065
Thréonine	0.014	0.3	0.0042	1.3	0.018	0.0013	0.3	0.00037	1.69	0.002
Water	20.97	0	0	1	20.97	22.32	0	0	1	22.32

As can be seen in Table 5, water represents the most preponderant relevant component from mass point of view, with values of about 20.97 and 22.32 kg/kgP, for input and output, respectively. As shown in Figure 5a this represents 85.4 and 87% for the overall process input and output, respectively. Therefore the raw materials come to just 15 and 8.97% for by-products and wastes, respectively and 3.90% for the mean product

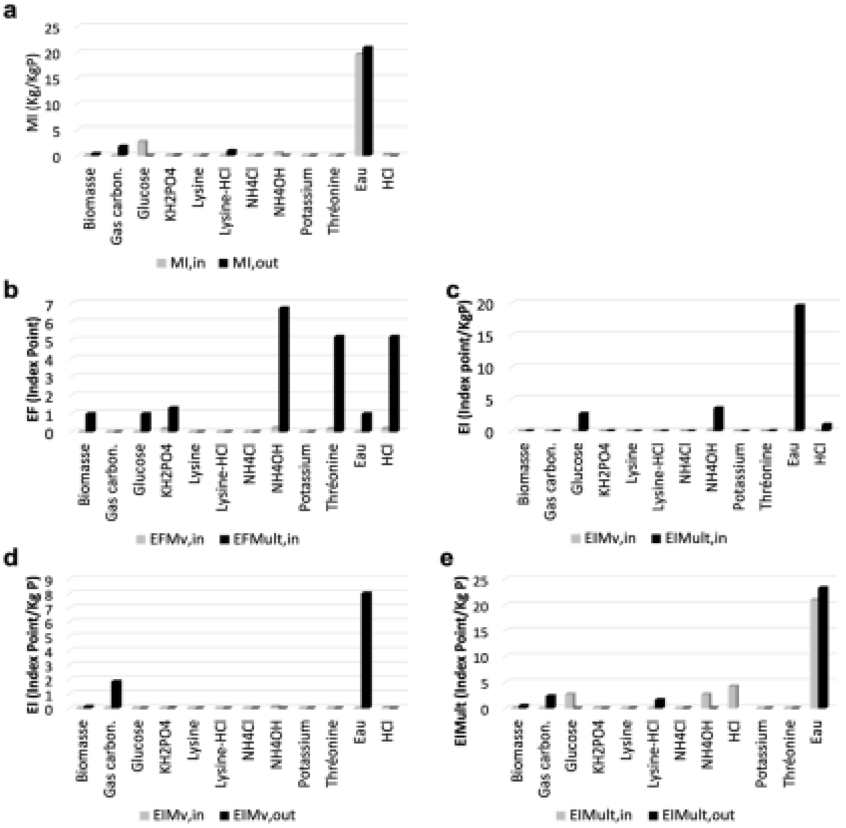

Figure 5. *Environmental indices evaluation of the Lysine production: (a) Inputs and outputs mass indices; (b) Input Environmental factor based on the average and multiplication calculation; (c) Inputs Environmental indexes; (d) Outputs Environmental indexes.*

Still from Figure 5a, it can be seen that Glucose is the second important input in terms of mass consumption with a value of 2.47 kg/kg P corresponding to a yield of 0.36, while carbon dioxide, biomass and lysine are for the output.

Beside glucose, water is then the main raw material, representing 10 times the amount of L-lysine-HCl produced. It can be seen that the output is higher than the input due to water generation during the fermentation step as confirmed by the water mass balance for the whole production process, represented in Figure 6. Moreover the water in the output effluents contains COD (chemical oxygen demand) as for the grading of the input water, the process water demand represents an equivalent of 500 inhabitants water consumption or 10 ha irrigation water demand.

Therefore it has been decided to recycle water to reduce its environmental impact and lysine to increase the production of L-lysine

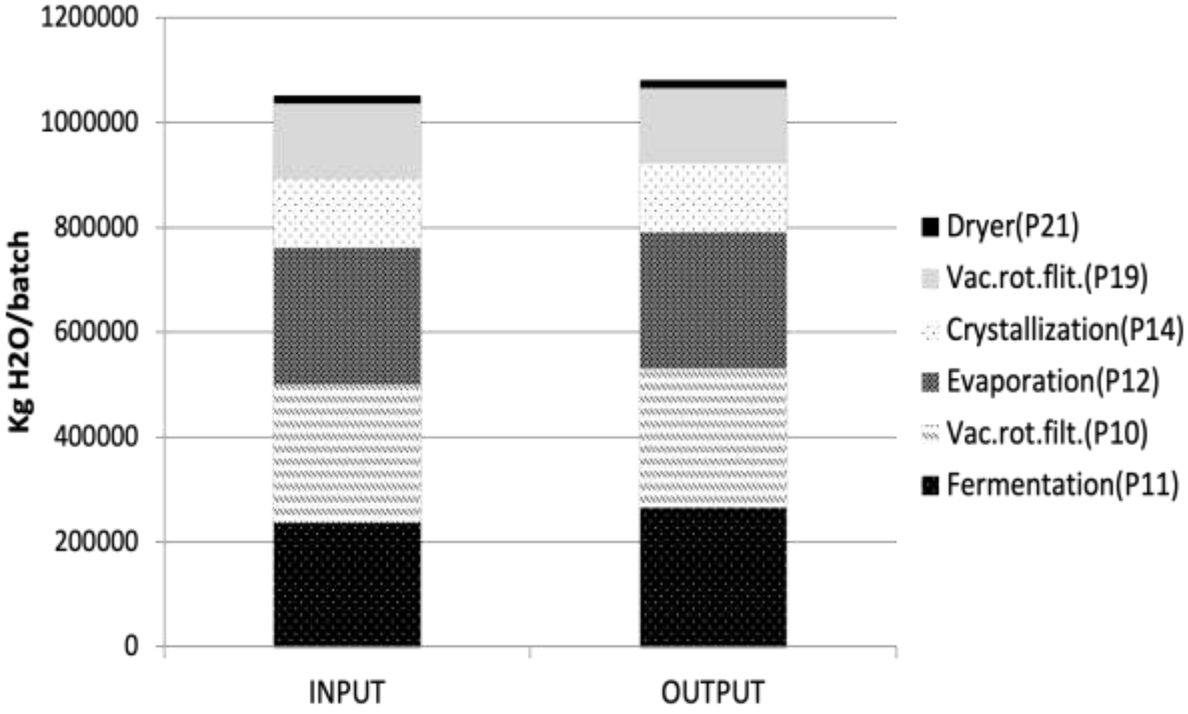

Figure 6. *Water mass balance for the L-lysine-HCl production.*

Since the output streams are various in the flowsheet, the impact will be evaluated for polluted streams from just rotary vacuum filter 1(P-10) and rotary vacuum filter 2 (P-19), containing respectively biomass and impurities.

Impurities stream contains substrate and nutrients in very small quantities comparatively with water amount so the mixture is much diluted, justifying their non-consideration as environmental pollutants. But biomass-2 stream shows that it is highly charged and needs treatment before disposal as shown in Table 6.

Tables 6. *Environmental characteristics of biomass and impurities streams.*

Parameter	**Stream Biomass-2**		**Stream Impurities**	
	Concentration	**Daily Throughputs**	**Concentration**	**Daily Throughputs**
TOC (mg C/l)	307 446.74	2 031.73	0.03	0.001
COD (kg O/d)	1 146 625.11	7 577.37	0.08	0.004
ThOD (kg O/d)	1 146 625.11	7 577.37	0.08	0.004
BODu (kg O/d)	1 054 895.10	6 971.18	0.06	0.003
BOD_5 (kg O/d)	717 328.67	4 740.40	0.05	0.003
TKN (kg N/d)	71 821.57	474.63	0.00	0.003
NH_3 (kg N/d)	71 821.57	474.63	0.00	0.000
NO_3/NO_2 (kg N/d)	0.00	0.00	0.00	0.000

TP (mg P/l)	12 600.28	83.27	0.00	0.000
TS (mg Slds/l)	630 013.80	4 163.39	0.07	0.004
TSS (mg Slds/l)	630 013.79	4 163.39	0.00	0.000
VSS (mg Slds/l)	567 012.41	3 747.05	0.00	0.000
DVSS (mg Slds/l)	567 012.41	3 747.05	0.00	0.000
TDS (mg Slds/l)	0.01	0.00	0.07	0.004
VDS (mg Slds/l)	0.01	0.00	0.07	0.004
DVDS (mg Slds/l)	0.01	0.00	0.07	0.004

BOD: biochemical oxygen demand; **COD:** chemical oxygen demand; **TOC:** total organic carbon; **TS:** total solids; **TSS**: total suspended solids; **TDS**: total dissolved solids; **TVS**: total volatile solids; **TKN**: total Kjeldahl nitrogen; **TP**: total phosphorus; **BOD_5**: five days biochemical oxygen demand, **ThOD**: theoretical oxygen demand; **VSS**: volatile suspended solids; **DVSS**: biodegradable volatile suspended solids; **VDS**: volatile dissolved solids; **DVDS**: biodegradable volatile dissolved solids.

Regarding the environmental index EIMult, unlike the environmental index EIMv, the effect of the mass is not cancelled as characterized by the mass index MI which is even closer to EIMult when the environmental impact is low. In fact the use of the material is important according to the sustainable development concept as illustrated by figure 5 (b, c, d and e).

The process environmental indices calculation shows clearly this difference. Table 7 presents the overall mass indices and environmental indices. EIM_{ult} process values are closer to MI than EIMv process values. Environmental index evaluation via multiplication takes into account of the impact categories of each component so it gets closer to the real situation as shown in Figures 7-a and b.

Table 7. *Process mass and environmental indices evaluation.*

$MI_{Process.in}$	$MI_{Process.out}$	$EIM_{VProcess.in}$	$EIM_{V\ Process.out}$	$EIM_{ult\ Process.in}$	$EIM_{ult\ Process.out}$
24.54669885	25.6161867	0.56061558	1.28701465	27.37652888	28.02526134

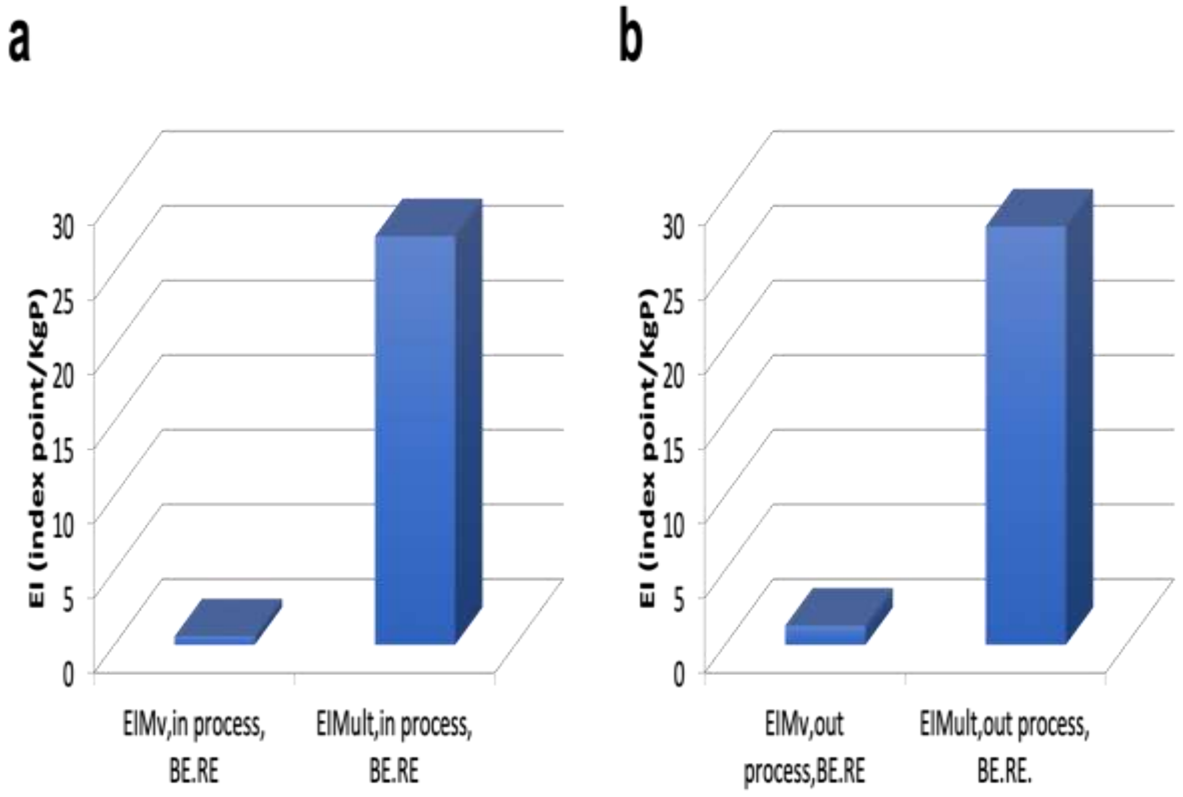

Figure 7. *Comparison between process environmental indexes based on multiplication (EIMult) and based on average (EIMv) for input and output respectively: (a) Input EIMv and EIMult; (b) Output EIMv and EIMult.*

IV Process flowsheet with recycling

As mentioned previously residual water from the evaporator and the dryer and after cooling is recycled to dissolve glucose, hence reducing the required fresh water quantity and its impact. The lysine is also separated from the biomass mixture leaving the rotary vacuum filter and that added in the crystallizer. The objective of this operation is to increase the lysine-HCl production and to eliminate lysine environmental impact. The new flowsheet is on figure 8.

Clearly from Table 8, the lysine recycling has improved its production rate but the overall yield is the same for both modes i.e. with and without recycling.

Table 8. *Recycling effect on $_L$-Lysine production.*

Before recycling		After recycling	
Production rate (kg/batch)	Overall Yield (%)	Production rate (kg/batch)	Overall yield (%)
21872.17	30.53	22324.71	30.53

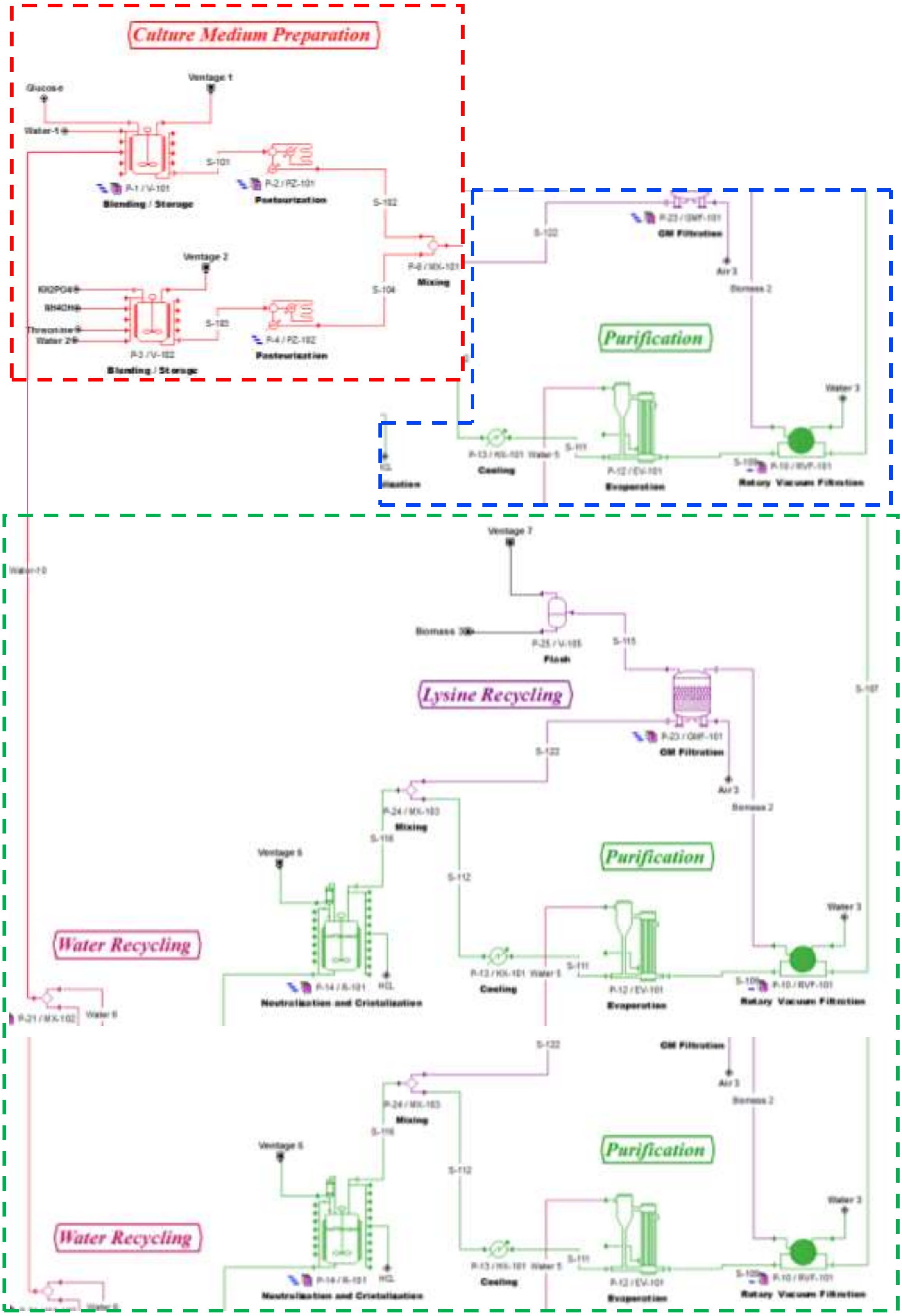

Figure 8. *Process flowsheet with water and lysine recycling.*

The environmental evaluation is shown in Table 9, where it can be seen that with L-lysine recycling, the quantity of HCl used for the crystallization has increased and fortunately the environmental impact of this material has not changed due to the increase of L-lysine production. Also the environmental impacts of the other inputs have been slightly reduced whereas the water Mult,in or Mult,out environmental indices have considerably decreased from 20.97 to 13.27 and from 22.32 to14.6 index point/kg P, respectively. In bioprocess the large amount of water is required for raw material dilution and as heat transfer agent. This consumption has its impact in water scarcity and degradation of ecosystems. That is why the recycling is morebenefit for the process. As it can be seen on figures 9 and 10 bellow, it is clear that process environmental impacts of the inputs and outputs after recycling are reduced.

Table 9. *Environmental assessment factors and indices after water and lysine recycling.*

Component	**Input**					**Output**				
	MI	EFv	EIMv	EFMult	EIMult	MI	EFv	EIMv	FMult	EIMult
Biomass	0.046	0	0	1	0.046	0.42	0.3	0.12	1.3	0.54
Gascarbon.	-	-	-	-	-	1.80	0.3	0.54	1.3	2.34
Glucose	2.69	0	0	1	2.69	4.47E-07	0.3	1.34E-07	1.3	5.82E-07
KH_2PO_4	0.045	0.3	0.0134	1.3	0.058	3.89E-05	0.65	2.53E-05	5.2	0.0002
Lysine	-	-		-	-	0	0	0	0	0
Lysine-HCl	-	-	-	-	-	1	0.3	0.3	1.69	1.69
NH_4Cl	-	-	-	-	-	0.0017	0.65	0.001	5.2	0.009
NH_4OH	0.515	0.65	0.335	5.2	2.68	0	1	0	16	0
HCl	0.201	1	0.201	4	0.803	0	0	0	0	0
Potassium	-	-	-	-	-	0.006	0	0	1	0.006
Thréonine	0.013	0.3	0.0041	1.3	0.018	0.001	0.3	0.0004	1.69	0.002
water	13.27	0	0	1	13.27	14.6	0	0	1	14.6

Table 10. *Comparison between process mass indices with and without recycling.*

Parameter	**Input**		**output**	
	Without recycling	**With recycling**	**Without recycling**	**With recycling**
EIMv	0 .56	0.55	0.99	0.97
EIMult	27.38	19.56	28.02	19.19

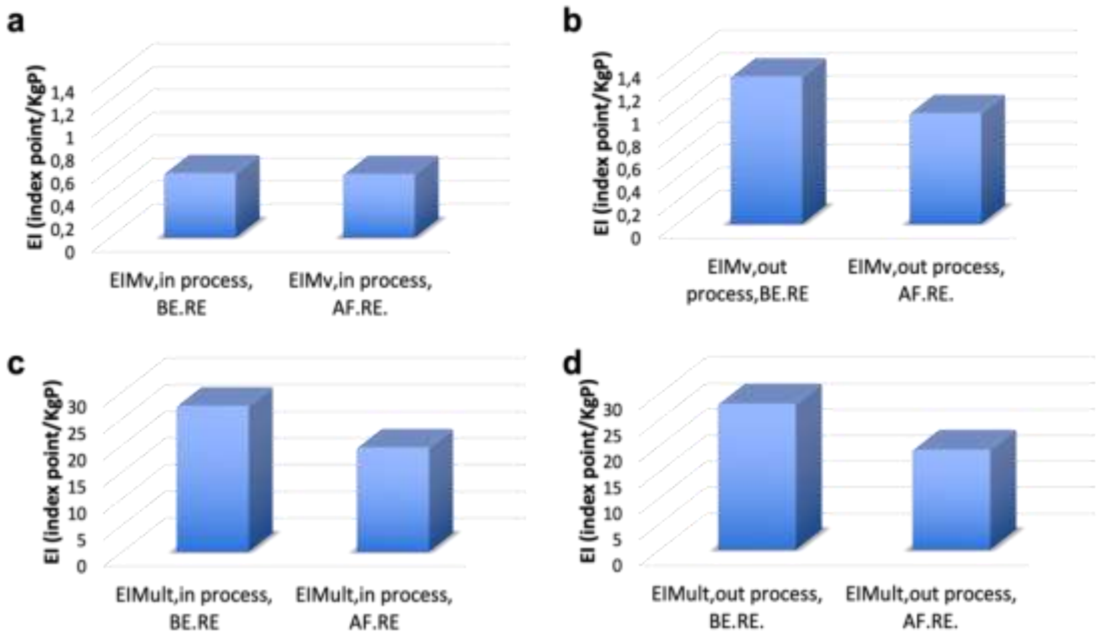

Figure 9. *Comparison between different process environmental indexes before recycling (BE.RE) and after recycling (AF.RE): (a) Input EIMv before and after recycling; (b) Output EIMv before and after recycling; (c) Input EIMult before and after recycling; (d) Output EIMult before and after recycling.*

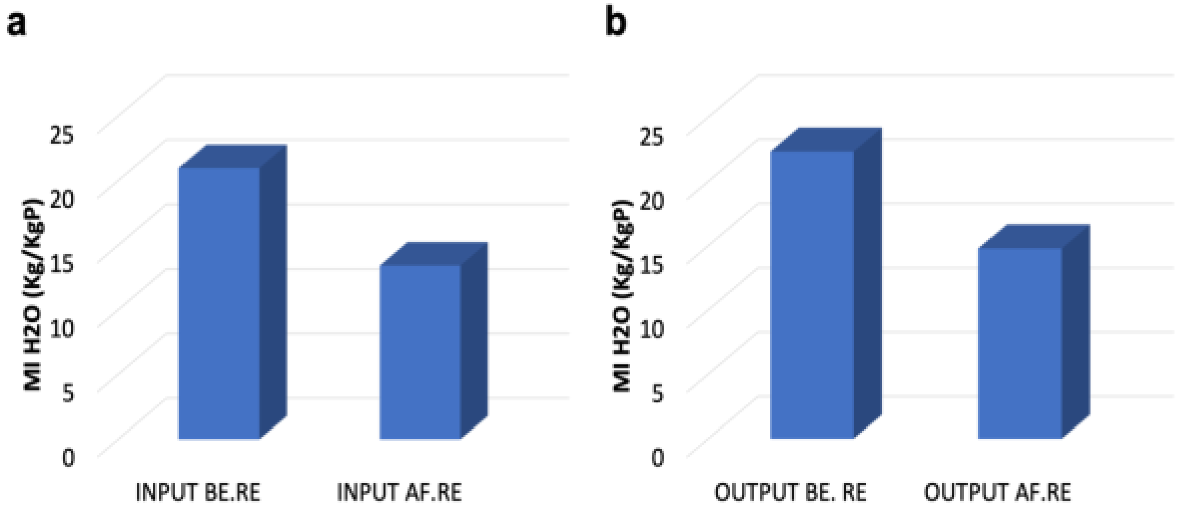

Figure 10. *Water environmental impact:*
(a) before and
(b) after recycling.

IV. Conclusion

Finally through this study one can see the ability of simulation to optimize existing processes or even to design new ones, taking into account not only the production yield and the financial investment return, but also considering key issues like the environmental, economical, and social impacts as well as the sustainability aspect, by means of a reliable software like Super Pro Designer® version 9.0**.**

In fact the obtained results have greatly contributed to achieve the main objective of the present study and which consists of an identification of the most influent part of the L-Lysine production process towards the environment, hence its impact. However the complexity of the problem is mainly due to many factors like the identification of the materials and steps which may effectively constitute an environmental load. This should be carried out at the very early stages of the process development, before even the project building since these environmental loads may be greatly reduced. Clearly this would reduce waste treatment costs as well avoid heavy penalties from the environmental authorities.

The obtained results clearly show that the proposed initiative consisting of including a process with the recycling of fresh water and Lysine greatly improved the production as well as the control of wastes and the safety of the process, as confirmed by the following main results:

- Water is the main raw material, representing 10 times the amount of L-lysine-HCl produced.
- Glucose is the second important input in terms of mass consumption with a value of 2.47 kg/kg P corresponding to a yield of 0.36, while carbon dioxide biomass and lysine are for the output.
- Water and Lysine recycling has reduced the process environmental impact and has increased the production of L-lysine-HCl.
- Recycling effectively has slightly reduced the environmental impacts of the other inputs whereas the water Mult, in or Mult, out environmental indices have considerably decreased from 20.97 to 13.27 and from 22.32 to14.6 index point/kg P, respectively.
- In bioprocess a large water amount is required for raw material dilution and also as a heat transfer agent. This has positive impacts as far as Water scarcity and Ecosystems degradation are concerned. Therefore the added recycling part to the process can be regarded as more than profitable.

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