**Applying entropy indexes to identify technological trajectories: second-generation bioethanol production**

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**ABSTRACT**

This paper uses the technological trajectories approach aiming to identify the presence of specialization in second-generation ethanol regarding the existence of modularity in the technological dimensions from the combination of types of biomass, enzymes and microorganisms. The methodology are applied in two steps, first where the entropy indexes are applied to variables obtained from patents, in correspondence of the dimensions related to delignification. Second, it was applied the NK model with the information of 304 patents of the sample has allowed to verify that the search processes in 'enzymatic hydrolysis for the production of lignocellulosic bioethanol' are still in the exploratory stage. These interdependencies and interactions between the various elements is what creates the trade-offs and, therefore, opens ways for different technological paths to emerge. The investigation of the evolution of the enzymes of commercial interest allowed to conclude that there is a concentration of the researches in the three cellulases and endo-beta-1,4-xylanase. That is, the most studied microorganisms are being directed to the synthesis of such enzymes. The interest in cellulases and xylanase makes sense if we consider that, from the polysaccharides present in the plant cell walls, cellulose is around 35 to 50% and that of the hemicellulose structures, xylan is one of the most representatives.

**Keywords**: entropy; trajectory; innovation; bioethanol; second-generation

**RESUMO**

Este artigo usa a abordagem das trajetórias tecnológicas com o objetivo de identificar a presença de especialização em etanol de segunda geração a respeito da existência de modularidade nas dimensões tecnológicas da combinação de tipos de biomassa, enzimas e microorganismos. A metodologia é aplicada em duas etapas, primeiro é aplicado o índice de entropia para as variáveis ​​obtidas a partir de patentes, em correspondência com as dimensões relacionadas com a deslignificação. Em segundo lugar, foi aplicado o modelo NK com a informação de 304 patentes da amostra e que permitiu verificar que os processos de busca em ‘hidrólise enzimática para a produção de bioetanol lignocelulósico’ ainda estão em fase exploratória. Estas interdependências e interações entre os vários elementos é o que cria os *trade-offs* e, portanto, abre caminhos para que diferentes rotas tecnológicas possam emergir. A investigação acerca da evolução das enzimas de interesse comercial permitiu concluir que existe uma concentração das pesquisas nas três celulases e endo-beta-1,4-xilanase. Isto é, os microrganismos mais estudados estão direcionados para a síntese de tais enzimas. O interesse em celulases e xilanases faz sentido se considerarmos que, a partir dos polissacáridos presentes nas paredes celulares de plantas, de celulose é de cerca de 35 a 50% e que as estruturas de hemicelulose, xilano é um dos mais representativos.

**Palavras-chave**: entropia; trajetória; inovação; bioetanol, segunda-geração

**Área ANPEC:** Área 9 - Economia Industrial e da Tecnologia

**Classificação JEL**: O33; Q16

## **Introduction**

The idea of technological trajectories (TT), pioneered by Dosi (1982) has contributed to clarify how a specific group of technologies evolves alongside a path, combining original new ideas with processes of learning, tacit knowledge and the results of market selection. However, there many relevant situations where is difficult to identify technological trajectories, with many alternatives that compete for a place in the future markets, meaning that competition had started many years before the first product or process have arrived in the market. This is the case of biotechnology (ORSENIGO, 1989) and certainly is the situation found in bioenergy (DAL POZ; SILVEIRA; MASAGO, 2013).

Since the 70s, with the two petroleum shocks, public and private agents from several countries have sought to develop, test and enable the production of alternative fuels to petroleum derivatives. Since then, there is a growing perception that the production of biofuels, i.e., fuels produced from renewable sources, has a great potential to replace the fossil-origin fuels (HLPE, 2013). The two most important countries in bioenergy, Brazil and USA, have been leading policies to promote the use of biofuel, defining Renewable Fuel Standards (RFS), combining incentives to technology innovation and doing market interventions, like mandates to anhydrous ethanol in gasoline and the promotion of flex fuel cars. These polices are based on the use of biomass, what at some extend compete with food, generating a huge debate about sustainability of biofuels in the last 15 years (ZILBERMAN et al., 2013).

The case of bioenergy, particularly the case of biofuels, emerges over the discussion about TT and the energy strategies:

1. There are already established technologies to produce ethanol, using saccharification and fermentation as an industrial development of traditional technologies;
2. New technologies frontiers compose of the use of new biomass sources (ranging from cellulosic material to waste), new biotechnology based processes and the possibilities to add value downstream, with alternatives “drop in” and “drop out” processes (WIELEN; BREUGEL, 2014);
3. There is an appeal to raise productivity and to reduce costs of biofuels and to improve the competitiveness of the bioenergy chain as a whole, motivating P&D efforts by public sector, private institutions and corporations (WILLEMS, 2015); and
4. Finally, some specific technologies, like enzymatic hydrolysis can be defined as a “focusing device” for the expanding of this technological frontier.

This paper applied a methodology originally developed by Frenken (2000) to identify the presence of specialization in second-generation ethanol regarding the existence of modularity in the technological dimensions from the combination of types of biomass, enzymes and microorganisms. Entropy indexes are applied to variables obtained from patents, in correspondence of the dimensions related to delignification. This presence in different locations generates the possibilities of persistence of technological variety (VENTURA et al., 2013) with specialization in the production of the ethanol, postponing the convergence of technologies. In the next section, the paper presents a panel with the distinct routes to produce ethanol and some forecast for technological alternatives. Section 3 presents the methodology (that is original in this field of economics of innovation), followed by the presentation of the model NK and respectively results and the final comments, respectively in the sections 4 and 5.

## **The technological variety to produce ethanol and the choice for enzymatic hydrolysis**

## **Characterizing technological variety in biofuels**

Biofuels is usually grouped in generations and have different development stages, generating technological alternatives, or alternative routes. These routes take into account the raw material and the conversion route employed in the production. In the case of bioethanol, there are three generations , but only the two most relevant are represented in Figure 1: one in advanced marketing stage (first-generation) and another in test stage and pilot plants, with some marketing plants installed recently (second-generation). Following the approach proposed by Frenken (2000), it is relevant to investigate the existence of technological variety and identify the presence of specialization of countries/regions in certain routes/technologies.

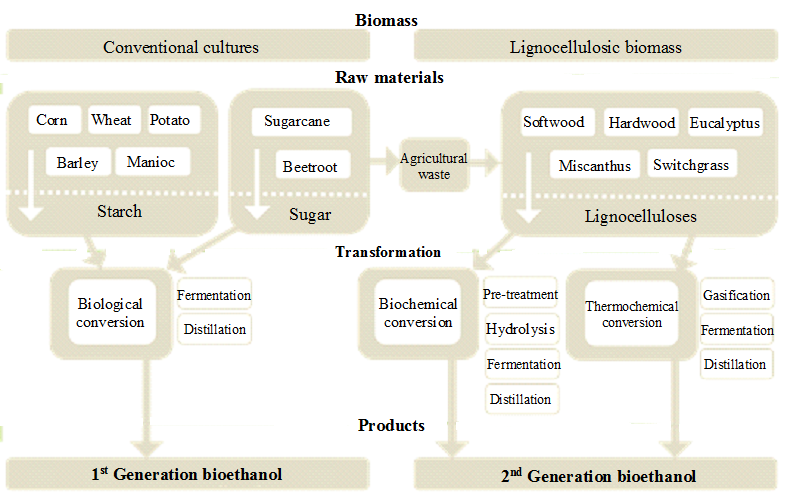


Figure 1. Routes for bioethanol production of first and second-generations

Source: Adapted and translated from HLPE (2013).

The so-called first-generation of bioethanol is produced from plants biomass, grains and cereals provided with directly fermentable carbohydrates (via biological conversion route), such as sugar and starch. The two main agricultural crops targeted for this purpose are the sugarcane, Brazil being the world's leading producer, and corn, whose production is led by the United States. But the potential of the second-generation of bioethanol (also called lignocellulosic bioethanol) is huge, since its raw material is the lignocellulosic biomass made up of complex carbohydrates, namely cellulose, hemicellulose and pectin (SOUZA, 2013).

Through biochemical conversion route[[1]](#footnote-1) these carbohydrates are converted into simple sugars that, when fermented, generate bioethanol. Cellulose, hemicellulose, and pectin, together with lignin, are the major structural components of the plants cell wall. Therefore, any plant material can be employed for the production of bioethanol, from crops dedicated to bioenergy (switchgrass, miscanthus, etc.) to forest and agricultural residues (SIMS et al., 2008). Before this promising source - biomass, some opportunities are opened to explore a wide range of raw materials, including lower costs than those used in the first-generation. This paper has the hypothesis that there is a huge room for the adoption of regional government policies that take into account the specificities of each country regarding the election of raw material(s) to be directed to the second-generation bioethanol production.

For biofuels to be produced on a large scale at competitive prices, however, it is necessary that its production reach similar cost levels or less than the cost of production of fossil fuels[[2]](#footnote-2). To date, the lignocellulosic bioethanol does not seem competitive, but there is much room for process improvements and therefore cost savings, since several technologies applied are in the early stages of development (HLPE, 2013).

The greater complexity involving the production of second-generation bioethanol, compared to the first-generation, requires that two additional production steps are inserted, namely, pretreatment and hydrolysis (Figure 1). The pretreatment step (or delignification) has as a purpose to open the cellular structure of the lignocellulosic material by breaking lignin in order to expose the cellulose and hemicellulose, so they can be hydrolyzed in the next step. The hydrolysis step (or saccharification) consists in breaking the polymer chains of cellulose, hemicellulose and pectin through insertion of water molecules. The steps of fermentation and distillation, whose techniques are widely mature in the production of the 1st generation bioethanol, consist, respectively, in the application of microorganisms capable of carrying out the conversion of simple sugars (glucose, for instance) in ethanol and the separation of ethanol from solid waste (SIMS et al., 2008; CANILHA et al., 2012). Nevertheless, despite the fermentation and distillation being techniques well mastered in the first-generation, the complexity of the production in the second-generation has required new forms of modularization of these productive stages (such as consolidated bioprocesses), yielding other boundaries of technological expansion for the steps until then considered mature (DAL POZ; SILVEIRA, 2015).

Both the pretreatment step and the hydrolysis step still carry too many uncertainties, there not being a leading technology or a dominant path. With respect to hydrolysis, literature mentions two routes that have been studied and have yet to show their technical and economic feasibility: enzymatic and acid. The first one requires the action of degrading enzymes produced by microorganisms, including certain species of bacteria, fungi and yeasts, whereas the latter requires the presence of acids (concentrated or diluted) (BONOMI, 2010).

The better usage of biomass generates incentives to the persistence of technology variety. Table 1, shows that the greatest content of hemicellulose/cellulose is in sugarcane straw. This case helps to understand the meaning of modularity and decomposition, formulated by Frenken (2000): if a mill makes a choice for using sugarcane straw for second-generation, she should have developed a system to collect the material, transport it and should have introduced changes in the reception system in the mills. Simultaneously, the mill have to face the highest content of lignin, meaning the need to treat waste and/or develop a system to process 5-carbon sugars. All these alternatives represent possible cost trajectories dependent on research results.

Table 1. Composition of Sugarcane Biomass

|  |  |  |  |
| --- | --- | --- | --- |
| **Composition** | **Biomass composition (wt %)** | | |
| **Sugarcane stalks** | **Sugarcane straw** | **Energy cane** |
| **Water** | 70.3 | 15.0 | 66.8 |
| **Sucrose** | 14.0 | 4.3 | 8.1 |
| **Reducing sugars** | 0.6 | 0.2 | 2.5 |
| **Fibers** | 12.7 | 77.9 | 21.3 |
| **Cellulose** | 6.0 | 32.4 | 10.0 |
| **Hemicellulose** | 3.5 | 24.8 | 5.9 |
| **Lignin** | 3.2 | 20.6 | 5.4 |
| **Others** | 2.4 | 2.6 | 1.3 |

Source: Junqueira (2015).

## **Scenarios for biofuels and the importance of enzymatic hydrolysis**

It is useful to summarize the results of Junqueira (2015) work, from a panel of experts in bioenergy to justify the methodological choice to focus on enzymatic hydrolysis (EH).

According to Junqueira (2015), it is possible to build short, medium and long-term scenarios for ethanol, combining the type of raw material (biomass) and the technologies. For instance, consider the Table 2, below with a scenario akin to Brazilian conditions to increase second-generation yields and recovery of by products (xylosis and others).

Table 2. Forecast for Second Generation Ethanol from sugarcane

|  |  |  |  |
| --- | --- | --- | --- |
| Description | Short Run | Medium Run | Long Run |
| 2G | All year: sugarcane bagasse  + straw | All year: energy cane bagasse | All year: energy cane bagasse |
| 1G2G | Season: sugarcane bagasse+ straw | Season: sugarcane bagasse+ straw  Off-season: energy cane bagasse | Season: sugarcane bagasse  + straw + energy cane bagasse  Off-season: energy cane bagasse |

Source: Junqueira (2015).

It is clear in the Table 2 that even with a rough classification, dealing only with the biomass sugarcane, there is a combination of convergence and variety. For greenfield mills dedicated to 2G, the biomass will converge to energy cane. For the mixed 1G2G mills, the economic advantage comes from of the drastic reduction of the off-season period (nowadays in 3 months).

A relevant result from the panel carried by Junqueira (2015) is the gain of 10% in the obtaining of glucose from cellulose in 10 years from now on and 10% more in the next 20 years, showing EH as a very promising alternative.

The Table 3, provides a curious insight to justify the relevance of EH. Brazil is leading in the number and in relevant scientific publications in EH, together with delignification, which is the closer research field. University of São Paulo, according to Bueno et al. (2015), is the prominent institution worldwide.

Regardless the type of lignocellulosic material (the options are many) that can be subjected to degradation, the cost and the efficiency of enzymatic hydrolysis are two of the factors that have restricted the wider use of biomass for conversion into bioethanol. Firstly, the production costs of enzymes are closely related to the productivity of microorganisms used, and different lignocellulosic microorganisms hold distinct abilities when it comes to diversity, the rate of synthesis and characteristics of the produced enzymes. On the other hand, the hydrolysis efficiency depends on the enzymes used and the synergic action between them. It is facing the challenge of reducing production costs and raise the efficiency of the enzymatic hydrolysis d) Finally, some specific technologies, like enzymatic hydrolysis can be defined as a “focusing device” for the expanding of this technological frontier process that many scientific and technological researches have been conducted.

Table 3. Most Relevant Institution by Scientific Research Areas on Sugarcane Worldwide

|  |  |  |  |
| --- | --- | --- | --- |
| Sub-area | Country with the most publication | Institution with the most publications | Most frequently cited |
| Enzymatic Hydrolysis | Brazil | University of São Paulo | 143 |
| Molecular Markers | Brazil | University of São Paulo | 204 |
| Delignification | Brazil | University of São Paulo | 510 |
| Genotypes | USA | United States Department of Agriculture | 204 |
| Enzymatic Conversion | Brazil | University of São Paulo | 510 |
| Genetic expression | China | Chinese Academy of Sciences | 270 |
| Nitrogen | Brazil | University of São Paulo | 274 |
| Photosynthesis | USA | United States department of agriculture | 257 |
| Pest Control | Brazil | University of São Paulo | 125 |

Source: Bueno et al. (2015).

Some micro-organisms are known as capable of synthesizing degrading enzymes of lignocellulosic material (called lignocellulosic enzymes), such as fungi of the genus *Trichoderma*, *Humicola*, *Aspergillus*, *Fusarium* and *Penicillium* and bacteria of the genus *Bacillus*, *Cellulomonas*, *Thermomonospora* and *Streptomyces*, to name a few examples (CANILHA et al., 2012). The lignocellulosic enzymes are subdivided in cellulases, hemicellulases and pectinases, requiring around 30 of these enzymes to fully convert biomass into monosaccharides (Table 4). The choice of enzymes (and their proportions) for the degradation of complex carbohydrates, however, depends on the adopted pretreatment process and the chemical composition of the biomass used (which varies according to the species, variety, type of fabric, growth conditions, maturity level of the plant, weather conditions, nutrients availability, etc.). On average, the biomass has 35-50% of cellulose, 20-35% of hemicellulose and small fractions of pectin (BRINK e VRIES, 2011).

As shown, there is a huge diversity of microorganisms and enzymes with a potential for lignocellulosic material degradation. There has been progress in the identification of these microorganisms and enzymes, but much research needs to be done to make them suitable for the production of bioethanol. It is expected that, with the advances in research and the maturing of the hydrolysis route, there is a bottleneck of the studied microorganisms, the gradual election of the fittest to the production of bioethanol and targeting certain enzymes, reducing the degree of uncertainty that involves the process.

It is also expected that the enzymatic pathway prevails, since their overall costs are lower (but still elevated, greatly increasing the costs of the process as a whole), the glycosidic yields are higher than those obtained by acid means, besides the fact to be able to count on the modern techniques of microbiology and genetic engineering to optimize the process.

Table 4. Lignocellulosic enzymes

|  |  |  |
| --- | --- | --- |
|  | **Enzyme** | **Degraded compound** |
| 1 | endo-beta-1,4-glucanase | cellulose |
| 2 | exo-beta-1,4-glucanase and cellobiohydrolase | cellulose |
| 3 | beta-glucosidase or cellobiase | cellulose |
| 4 | exo-beta-1,4-glucanase or celodextrinase\*\* | cellulose |
| 5 | endo-beta-1,4-xylanase | hemicellulose |
| 6 | beta-1,4-xylosidase | hemicellulose; pectin |
| 7 | endo-beta-1,4-mannanase | hemicellulose |
| 8 | beta-mannosidase | hemicellulose |
| 9 | endo-beta-1,4-glucanase specific for xyloglucan | hemicellulose |
| 10 | alpha-L-arabinofuranosidase | hemicellulose; pectin |
| 11 | arabinoxylan arabinofuranohydrolase | hemicellulose |
| 12 | alpha-glucuronidase | hemicellulose |
| 13 | alpha-xylosidase | hemicellulose |
| 14 | alpha-L-fucosidase | hemicellulose |
| 15 | alpha-1,4-galactosidase | hemicellulose |
| 16 | 1,4-beta-galactosidase | hemicellulose; pectin |
| 17 | acetyl xylan esterase | hemicellulose |
| 18 | feruloil esterase | hemicellulose; pectin |
| 19 | endo-polygalacturonase | pectin |
| 20 | exo-polygalacturonase | pectin |
| 21 | endo-rhamnogalacturonase | pectin |
| 22 | exo-rhamnogalacturonase | pectin |
| 23 | endo-xilogalacturonase | pectin |
| 24 | exo xilogalacturonase | pectin |
| 25 | alpha-L-rhamnosidase | pectin |
| 26 | glucuronyl hydrolase unsaturated | pectin |
| 27 | rhamnogalacturonan hydrolase unsaturated | pectin |
| 28 | pectin lyase | pectin |
| 29 | pectate lyase | pectin |
| 30 | rhamnogalacturonan lyase | pectin |
| 31 | endo-arabinanase | pectin |
| 32 | exo-arabinanase | pectin |
| 33 | endo-beta-1,4-galactanase | pectin |
| 34 | pectin methyl esterase | pectin |
| 35 | rhamnogalacturonan acetyl esterase | pectin |

Source: Own preparation, from Brink e Vries (2011).

## **Methodology**

## **Delimitation of the sample**

Several papers are based on information contained in patent documents to investigate phenomena related to innovation economy (KRAFFT et al., 2009; VENTURA et al., 2013; EPICOCO, 2013; DAL POZ; SILVEIRA; MASAGO, 2013; SOUZA, 2013). The sample of patents related to the theme ‘enzymatic hydrolysis to produce lignocellulosic bioethanol’ for the investigation of technological paths was extracted from the base of Derwent Innovations Index during the period of 1970 and 2014.

For the delimitation of the sample, IPCs (International Patents Classification) were previously selected through the tool ‘IPC STATS Search’, available from the WIPO (World Intellectual Property Organization) website. From the inclusion of one or a combination of keywords, the tool displays IPCs most related to the words proposed. Terms used with this tool were:

* *biomass*, *lignocellulosic*, *cellulose*, *hemicellulose*, *ethanol*, *fuel*, *biochemical conversion*, *bioconversion*, *enzymatic hydrolysis*, *hydrolysis*, *enzyme*, *fermentation*, *saccharification*, *"separate hydrolysis and fermentation"*, *"simultaneous saccharification and fermentation"*, *"simultaneous saccharification and cofermentation"* and *"consolidated bioprocessing"*.

It was observed, with the inclusion of these terms, a predominance of patents in subclasses C12P (*Fermentation or enzyme-using processes to synthesize a desired chemical compound or composition or to separate optical isomers from a racemic mixture*) and C12N (*Microorganisms or enzymes; compositions thereof; propagating, preserving, or maintaining microorganisms; mutation or genetic engineering; culture media*).

The investigation of the subgroups (lower level of disaggregation of IPC) belonging to C12P and C12N subclasses resulted in the selection of 44 IPCs codes of interest (Table 5).

With the filters on the level of the subgroups previously defined, went up to the second step of the methodological route: the construction of the patents sample. The base used was the Derwent Innovations Index, owned by Thomson Reuters and gathers information from 47 patent issuing authorities, including the North American (USPTO), European (EPO) and Japanese (JPO) Offices, and the base of the World Organization of Intellectual Property (PATENTSCOPE). The choice for this base was due to the fact that it covers many patent offices.

For the construction of the sample, 44 IPCs code selected by the search filter were used:

* IP=(C12P-007/06 **OR** C12P-007/08 **OR** C12P-007/10 **OR** C12P-007/14 **OR** C12P-019/00 **OR** C12P-019/02 **OR** C12P-019/04 **OR** C12P-019/12 **OR** C12P-019/14 **OR** C12P-039/00 **OR** C12N-001/00 **OR** C12N-001/12 **OR** C12N-001/13 **OR** C12N-001/14 **OR** C12N-001/15 **OR** C12N-001/16 **OR** C12N-001/18 **OR** C12N-001/19 **OR** C12N-001/20 **OR** C12N-001/21 **OR** C12N-001/22 **OR** C12N-009/00 **OR** C12N-009/02 **OR** C12N-009/04 **OR** C12N-009/14 **OR** C12N-009/24 **OR** C12N-009/42 **OR** C12N-015/01 **OR** C12N-015/02 **OR** C12N-015/03 **OR** C12N-015/04 **OR** C12N-015/05 **OR** C12N-015/09 **OR** C12N-015/10 **OR** C12N-015/11 **OR** C12N-015/52 **OR** C12N-015/53 **OR** C12N-015/54 **OR** C12N-015/55 **OR** C12N-015/56 **OR** C12N-015/63 **OR** C12N-015/80 **OR** C12N-015/81 **OR** C12N-015/82)

As IPCs selected not only involve the topic of interest, sample cuts were performed through keywords. First, it was decided to apply the terms used for the selection of IPCs, because of the adherence with the research theme ‘enzymatic hydrolysis to produce lignocellulosic bioethanol’. The search filter used was:

* biochemical conversion OR bioconversion OR hydrolysis OR saccharification OR biomass OR lignocellulose OR lignocellulosic OR cellulose OR cellulosic OR hemicellulose OR enzyme OR enzymes OR enzymatic OR ethanol OR bioethanol OR fuel OR biofuel

Table 5. Selected IPCs (subgroups level)

|  |  |  |
| --- | --- | --- |
| **Subgroup** | | **Description** |
| C12P7 | /06 | Preparation of oxygen-containing organic compounds; containing a hydroxy group; acyclic; Ethanol, i.e. non-beverage |
| /08 | Preparation (…); produced as by-product or from waste or cellulosic material substrate |
| /10 | Preparation (…); produced as by-product or (…); substrate containing cellulosic material |
| /14\* | Preparation (…); Multiple stages of fermentation; Multiple types of micro-organisms or reuse for micro-organisms |
| C12P19 | /00 | Preparation of compounds containing saccharide radicals |
| /02 | Preparation (…); Monosaccharides |
| /04 | Preparation (…); Polysaccharides |
| /12\* | Preparation (…); Disaccharides |
| /14 | Preparation (…); produced by the action of a carbohydrase |
| C12P39 | /00\* | Processes involving micro-organisms of different genera in the same process, simultaneously |
| C12N1 | /00 | Micro-organisms; Compositions thereof; Processes of propagating, maintaining or preserving micro-organisms or compositions thereof; Processes of preparing or isolating a composition containing a micro-organism; Culture media therefor |
| /12 | Micro-organisms (...); Unicellular algae; Culture media therefor |
| /13\* | Micro-organisms (...); Unicellular algae (…); modified by introduction of foreign genetic material |
| /14 | Micro-organisms (...); Fungi; Culture media therefor |
| /15 | Micro-organisms (...); Fungi; modified by introduction of foreign genetic material |
| /16 | Micro-organisms (...); Fungi; Yeasts; Culture media therefor |
| /18 | Micro-organisms (...); Fungi; Yeasts (…); Baker's yeast; Brewer's yeast |
| /19 | Micro-organisms (...); Fungi; Yeasts (…); modified by introduction of foreign genetic material |
| /20 | Micro-organisms (...); Bacteria; Culture media therefor |
| /21 | Micro-organisms (...); Bacteria (…); modified by introduction of foreign genetic material |
| /22 | Micro-organisms (...); Processes using, or culture media containing, cellulose or hydrolysates thereof |
| C12N9 | /00 | Enzymes; Proenzymes; Compositions thereof; Processes for preparing, activating, inhibiting, separating, or purifying enzymes |
| /02\* | Enzymes (…); Oxidoreductases |
| /04 | Enzymes (…); Oxidoreductases; acting on CHOH groups as donors |
| /14 | Enzymes (…); Hydrolases |
| /24 | Enzymes (…); Hydrolases; acting on glycosyl compounds |
| /42 | Enzymes (…); Hydrolases (…); acting on beta-1, 4-glucosidic bonds, e.g. cellulose |
| C12N15 | /01\* | Mutation or genetic engineering; DNA or RNA concerning genetic engineering, vectors or their isolation, preparation or purification; Use of hosts therefor; Preparation of mutants without inserting foreign genetic material therein; Screening processes therefor |
| /02\* | Mutation (…); Preparation of hybrid cells by fusion of two or more cells |
| /03\* | Mutation (…); Preparation of hybrid cells by fusion of two or more cells; Bacteria |
| /04\* | Mutation (…); Preparation of hybrid cells by fusion of two or more cells; Fungi |
| /05 | Mutation (…); Preparation of hybrid cells by fusion of two or more cells; Plant cells |
| /09 | Mutation (…); Recombinant DNA-technology |
| /10 | Mutation (…); Recombinant (…); Processes for the isolation, preparation or purification of DNA or RNA |
| /11\* | Mutation (…); Recombinant (…); DNA or RNA fragments; Modified forms thereof |
| /52 | Mutation (…); Recombinant (…); DNA (…); Modified (…); Genes encoding for enzymes or proenzymes |
| /53\* | Mutation (…); Recombinant (…); DNA (…); Modified (…); Genes (…); Oxidoreductases |
| /54 | Mutation (…); Recombinant (…); DNA (…); Modified (…); Genes (…); Transferases |
| /55 | Mutation (…); Recombinant (…); DNA (…); Modified (…); Genes (…); Hydrolases |
| /56 | Mutation (…); Recombinant (…); DNA (…); Modified (…); Genes (…); Hydrolases; acting on glycosyl compounds |
| /63 | Mutation (…); Recombinant DNA-technology; Introduction of foreign genetic material using vectors; Vectors; Use of hosts therefor; Regulation of expression |
| /80 | Mutation (…); Recombinant (…); Introduction of foreign (…); Vectors or expression systems specially adapted for eukaryotic hosts; for fungi |
| /81\* | Mutation (…); Recombinant (…); Introduction of foreign (…); Vectors (…); for fungi; for yeasts |
| /82 | Mutation (…); Recombinant (…); Introduction of foreign (…); Vectors (…); for plant cells |

Source: Own preparation, from the descriptions of the International Patent Classification, available at <http://www.wipo.int/classifications/ipc/en/>.

\*The 12 subgroups marked with an asterisk were not found by the tool ‘IPC STATS Search’. However, they were incorporated because they were considered related to the topic.

From the sample cut by terms adherent to the subject, a second sample cut was performed with a view to obtain patents related to micro-organisms used in the enzymatic hydrolysis processes for the production of bioethanol second-generation. The search filter was built incorporating, *a priori*, bacteria and fungi (in the genus level) known to be used in these processes, as well as general terms in order to capture other micro-organisms not discriminated directly by the filter. The filter used was:

* Acetivibrio OR Bacillus OR Bacteroides OR Cellulomonas OR Clostridium OR Erwinia OR Ruminococcus OR Streptomyces OR Microbispora OR Thermomonospora OR (genus AND bacterium) OR (genus AND bacteria) OR (genus AND bacteria\*) OR (genera AND bacterium) OR (genera AND bacteria) OR (genera AND bacteria\*) OR (specie AND bacterium) OR (specie AND bacteria) OR (specie AND bacteria\*) OR (species AND bacterium) OR (species AND bacteria) OR (species AND bacteria\*) OR (bacterium OR bacteria) OR Aspergillus OR Penicillium OR Phanerochaete OR Schizophyllum OR Sclerotinia OR Trichoderma OR (genus AND fungus) OR (genus AND fungi) OR (genus AND fung\*) OR (genera AND fungus) OR (genera AND fungi) OR (genera AND fung\*) OR (specie AND fungus) OR (specie AND fungi) OR (specie AND fung\*) OR (species AND fungus) OR (species AND fungi) OR (species AND fung\*) OR (fungus OR fungi)

Also from the sample cut by terms adherent to the subject, another sample cut was performed with a view to obtain patents related to raw materials used in the second-generation bioethanol production processes. As was done previously, the search filter incorporated, *a priori*, raw materials knowingly used, as well as general terms, in order to find patents involving other raw materials not directly discriminated by the filter. The filter used was:

* swichgrass OR miscanthus OR prairie grass OR eucalyptus OR softwood OR hardwood OR willow OR poplar OR spruce OR loblolly pine OR pinus taeda OR cane bagasse OR bagasse OR corn stover OR stover OR husk OR stalk OR treetop OR treetops OR branch OR branches OR perennial grass OR perennial grasses OR residue OR residues OR waste OR forest

In order to define a sample with the lowest possible noise level, the sample cuts from micro-organisms and from the raw materials were crossed, resulting in a total of 4,118 patents to be studied one by one (Table 6). Aggregation was made because, as the Boolean operator used in the sample cuts was union (OR), it is very likely that the base search engine of the Derwent Innovations Index has recovered information from patents that have at least one of the applied terms and these terms alone does not necessarily involve the subject covered in this article.

Table 6. Number of patents per period and per applied search filter

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Period** | **Filter by IPCs** | **Filter by adherent terms** | **Filter by micro-organisms** | **Filter by raw materials** | **Micro-organisms crossing X raw materials** |
| 1970-1979 | 673 | 268 | 89 | 44 | 9 |
| 1980-1989 | 10,566 | 3,852 | 1,368 | 499 | 168 |
| 1990-1999 | 35,553 | 8,831 | 2,634 | 1,360 | 454 |
| 2000-2009 | 98,852 | 29,832 | 9,364 | 6,758 | 2,311 |
| 2010-2014 | 48,012 | 15,827 | 5,631 | 3,266 | 1,176 |
| Total | 193,656 | 58,610 | 19,086 | 11,927 | 4,118 |

Source: Own preparation, from the use of *Derwent Innovations Index* base, available at <http://www.periodicos.capes.gov.br/>. Access on: May 14, 15, 16, 19, and 20, 2014.

The patent analysis, one by one, resulted in the selection of 304 patents (a total of 4,118) related to micro-organisms and enzymes used in the enzymatic hydrolysis process for the production of lignocellulosic bioethanol (Figure 2).

Figure 2. Patents selected for the sample

Source: Own preparation, from the sample cut obtained with the patents information from the base Derwent Innovations Index, available at <http://www.periodicos.capes.gov.br/>.

## **Application of the model NK**

The option to apply the NK-model to identify the technological paths for enzymatic hydrolysis is due, initially, to the idea that technological innovations[[3]](#footnote-3) should be thought of as complex systems containing several elements that are interdependent and interact in various ways. These interdependencies and interactions between the various elements is what creates the trade-offs and, therefore, opens ways for different technological paths to emerge. It is expected that technological paths that will be formed in the enzymatic hydrolysis are the result of optimal combinations between the three dimensions ‘content-microorganism-enzyme’ studied.

Around the enzymatic hydrolysis route for the production of lignocellulosic bioethanol, there are many uncertainties related to the enormous diversity of microorganisms and enzymes with potential for lignocellulosic material degradation. There has been progress in the identification of these microorganisms and enzymes, but much research needs to be done to make them suitable for the production of bioethanol. It is expected that, with the advances in research and the maturing of the hydrolysis route, there is a bottleneck of the studied microorganisms, the gradual election of the fittest to the production of bioethanol and targeting certain enzymes, reducing the degree of uncertainty that involves the process, according to the establishment of optimal combinations, mainly among microorganisms and enzymes.

Accordingly, NK model assists the work, to the extent that it allows for the combinations of the kinds of research being undertaken and the microorganisms and enzymes that are being studied to be detected. To these combinations that are formed, they are called standards of expertise and these standards of expertise that, when are maintained over time, give the direction of the technological paths for enzymatic hydrolysis to the production of lignocellulosic bioethanol.

In order to apply the NK model, were drawn from the 304 patents three categories of information, namely, the content (nature of the research that has been carried out and that is intended to protect through the patent), the used microorganism and enzyme of interest. The analysis allowed to classify the 304 patents in 7 content categories (dimension X), 83 categories of microorganisms genus (dimension Y) and 34 categories of enzymes (dimension Z), as shown in Table 7. The number preceding each possibility represents the index used for the calculation of the NK model indicators.

Table 7. Dimensions of 304 patents for the application of NK model

|  |  |
| --- | --- |
| **Category** | **Description** |
| X: content | (1) culture of a micro-organism; definition of parameters for the culture medium; (2) OGM;  (3) expression of genes encoding lignocellulosic enzymes in yeast / plants / micro-organisms; (4) discovery of a new micro-organism; (5) isolating, cloning, modifying the gene encoding the enzyme; (6) modification of the enzyme amino acid sequence; (7) preparation of the enzyme mix |
| Y: micro-organism | (1) Acidothermus; (2) Acremonium; (3) Agaricus; (4) Anaerocellum; (5) Aspergillus; (6) Aureobasidium; (7) Azospirillum; (8) Bacillus; (9) Bifidobacterium; (10) Butyrivibrio; (11) Caldocellum; (12) Candida; (13) Cellulomonas; (14) Cellulophaga; (15) Cellvibrio; (16) Chaetomium; (17) Chloroflexi; (18) Chrysosporium; (19) Clostridium; (20) Cochliobolus; (21) Coniothyrium; (22) Coptotermes; (23) Deinococcus; (24) Erwinia; (25) Eubacterium; (26) Eupenicillium; (27) Eurotium; (28) Fusarium; (29) Galactomyces; (30) Geobacillus; (31) Geotrichum; (32) Gibberella; (33) Gliocladium; (34) Humicola; (35) Irpex; (36) Kluyveromyces; (37) Lecythophora; (38) Leuconostoc; (39) Limnoriidae; (40) Melanocarpus; (41) Meripilus; (42) Myceliophthora; (43) Myriococcum; (44) Myrothecium; (45) Nasutitermes; (46) Neocallimastix; (47) Neosartorya; (48) Neurospora; (49) Nodulisporium; (50) Ochrobactrum; (51) Orpinomyces; (52) Paenibacillus; (53) Penicillium; (54) Phanerochaete; (55) Piromyces; (56) Pleurotus; (57) Pseudoalteromonas; (58) Pseudomonas; (59) Psychrobacter; (60) Pyrococcus; (61) Rhizopus; (62) Rhodothermus; (63) Robillarda; (64) Saccharomycopsis; (65) Saccharophagus; (66) Schizochytrium; (67) Schizophyllum; (68) Scytalidium; (69) Sphingobacterium; (70) Streptomyces; (71) Talaromyces; (72) Termitomyces; (73) Thermoanaerobacterium; (74) Thermoascus; (75) Thermobifida; (76) Thermotoga; (77) Thielavia; (78) Trichoderma; (79) Verticillium; (80) Vibrio; (81) Volvariella; (82) Xylanimonas; (83) n.i. |
| Z: enzyme | (1) endo-beta-1,4-glucanase; (2) exo-beta-1,4-glucanase; (3) beta-glucosidase; (4) endo-beta-1,4-xylanase; (5) beta-1,4-xylosidase; (6) endo-beta-1,4-mannanase; (7) beta-mannosidase; (8) xyloglucanase (endo-beta-1,4-glucanase specific for xyloglucan); (9) alpha-L-arabinofuranosidase; (10) alpha-glucuronidase; (11) alpha-xylosidase; (12) alpha-L-fucosidase; (13) alpha-1,4-galactosidase; (14) beta-1,4-galactosidase; (15) acetyl xylan esterase; (16) feruloil esterase; (17) endo-polygalacturonase; (18) exo-polygalacturonase; (19) endo-rhamnogalacturonase; (20) exo-rhamnogalacturonase; (21) alpha-L-rhamnosidase; (22) unsaturated glucuronyl hydrolase; (23) pectin lyase; (24) pectate lyase; (25) rhamnogalacturonan lyase; (26) endo-arabinanase; (27) exo-arabinanase; (28) endo-beta-1,4-galactanase; (29) pectin methyl esterase; (30) rhamnogalacturonan acetyl esterase; (31) cellulase; (32) hemicellulase; (33) pectinase; (34) n.i. |

Source: Own preparation, from the sample cut obtained with the patents information from the base *Derwent Innovations Index*, available at <http://www.periodicos.capes.gov.br/>.

Since the quantity of categories for each dimension is high, the number of all possible combinations of ‘content-micro-organism-enzyme’ is also large. It is expected that, with the development of the enzymatic hydrolysis route, some of these combinations are set as great places, i.e., that certain types of searches are directed toward a group of micro-organisms that potentially synthesize a set of enzymes as most efficient.

To evaluate the development of enzymatic hydrolysis route in the sense if it has been an increasing degree of specialization in the relationship ‘content-micro-organism-enzyme’, namely, if paths have emerged relating to specific triads ‘content-micro-organism-enzyme’, was calculated the measure of mutual three-dimensional information. This indicator shows the mutual dependence between the variables of interest, that is, to what extent knowing the behavior of one of the variables can allow them to know the behavior of the others. On the one hand, if the variables are independent, knowing the behavior of one of them does not help in predicting the behavior of others and, therefore, the mutual information will be zero. But if, on the other hand, the behavior of a variable is a function of the behavior of the others, it is possible to foreseen it, and the mutual information signals this possibility when its value is greater than zero. The indicator of mutual three-dimensional information was calculated as follows:

|  |  |
| --- | --- |
|  | (1) |

The indicator was calculated based on the distribution of relative frequencies of triple combinations (*p*xyz) and the distribution of individual relative frequencies of each dimension (*p*x, *p*y e *p*z). In the absence of an expertise, the joint probability (*p*xyz) is equal to the multiplication of the individual probabilities (*p*x.*p*y.*p*z) and, therefore, T (X, Y, Z) takes the value zero. The presence of some degree of specialization makes T(X, Y, Z) to take positive values, and the higher this index, the higher the degree of expertise.

To accomplish this temporal analysis, however, the information of the patents were divided into groups of five years (based on date of filing) and applied the sliding window method year by year. Thus, the indicator was calculated for 36 periods: period 1 from 1975-1979, period 2 from 1976-1980, period 3 from 1977-1981, until period 36 from 2010-2014. Figure 3 shows the values of T(X, Y,Z), each year of the x-axis referring to the last year of each period. For example, the first value in the graph is 1979 and represents the value of T for the period of 1975-1979.

Figure 3. Values of T(X, Y, Z) of the patents from 1975-2013

Source: Own preparation.

What can be seen, from the illustrated graphic evolution, it is that there was an increase in the degree of specialization between dimensions during the period analyzed. It is observed that, until the mid-80s, there was a substantial increase in the indicator, followed by a significant decrease until 1990 and a new growth, and from 1995, the mutual information indicator remains stable and high, which indicates that a set of searches may be being directed to a set of micro-organisms which, in turn, may be being directed to a set of enzymes. Nevertheless, we must be extremely cautious in concluding anything from Figure 3 for two reasons. Firstly, because the degree of specialization indicator only responds if ‘it has been’ or ‘it has been not’ a similar behavior and “dependent”, in terms of frequency of occurrences among the variables, within a period of time. Secondly, because the indicator does not respond in what dimensions pairs that degree of expertise is manifesting.

To answer from which pairs of dimensions the degree of expertise is manifesting over time, it is necessary to examine the extent of mutual bi-dimensional information from among ‘content-micro-organism’, ‘content-enzyme’ and ‘micro-organism-enzyme’. This indicator reveals the presence (or not) of dependence between two variables of interest and the reasoning of the analysis is the same as for the three-dimensional case. The formulas of mutual information measure for each pair of dimensions and the respective graphs (Figure 4, 5 and 6) are described below, where T(X, Y) refers to the pair ‘content-micro-organism’, T(X, Z) to the pair ‘content-enzyme’ and T(Y, Z) to the pair ‘micro-organism-enzyme’. The calculations followed the same previous method, with the patents separated into groups of five years (based on the date of filing) and with a sliding window, year by year.

|  |  |
| --- | --- |
|  | (2) |
|  | (3) |
|  | (4) |

Figure 4. Values of T(X, Y) of the patents from 1975-2013

Source: Own preparation.

Regarding the pair ‘content-micro-organism’ (Figure 4), which can be seen is that there was an increase in the degree of expertise between the two dimensions over the analysis period. It is observed that, until 1987, there was a substantial increase in the indicator, followed by a significant decrease until 1990 and by a new growth, and from 1995, the mutual information indicator remains stable and with high levels. Analyzing all information on the screen period (1975-2013), it is possible to state that, during the 70s and the 80s, surveys were based greatly on ‘defining parameters of the culture medium’, to be optimized for the synthesis of enzymes. The microorganism most studied for this purpose was the fungus from the genus *Trichoderma* (during the 70s and the 80s), followed by the fungus from the genus *Penicillium* (during the first half of the 90s). In the 90s, 2000 and 2010, specifically from 1995, researches related to the ‘culture of micro-organism’ lose protagonism and are gaining increasing importance the researches based on biotechnological techniques, such as ‘isolation, cloning, modification of the gene synthesizing the enzyme’, ‘GM’ and ‘modification of the enzyme amino acid sequence’.

While in the first half of the 90s there was a predominance of researches related to the ‘culture of micro-organism’ with the fungus of the genus *Penicillium*, in the second half of the 90s, the researches directed predominantly to ‘isolation, cloning, modification of the gene synthesizing the enzyme’, but without a significant targeting to any micro-organism. Also regarding the research of ‘isolation, cloning, modification of the gene synthesizing the enzyme’, the decades of 2000 and 2010 followed the same trend of the second half of the 90s, without a significant expertise in specific microorganisms. Rather, a variety of microorganisms being searched were detected, indicating an effort to understand and deeply explore new micro-organisms.

The decades of 2000 and 2010 are marked by a *boom* in the number of patents related to the theme ‘enzymatic hydrolysis to produce lignocellulosic ethanol’. 124 patents were found for each period ahead to 36 patents found for the previous decade (see Figure 2). This result was accompanied by a change in the direction of the researches: the content ‘modification of the enzyme amino acid sequence’ starts to become more important in the last two decades studied, with 43 patents in each period. In the decade of 2000, there was a specialization of such research in two microorganisms, namely, the fungus *Trichoderma* and the bacterium *Bacillus*.

Finally, in the decade of 2010, it was verified the specialization among the research ‘modification of the enzyme amino acid sequence’ and the fungus *Trichoderma*, and from this kind of research and the fungus *Myceliophthora*. Another striking phenomenon that deserves attention during this decade has been the growth in the number of patents related to the researches with OGM, going from 13 to 26 patents between the periods of 2000 and 2010. Of the 26 patents, 8 directed to the bacteria *Clostridium* and 5 directed to the fungus *Trichoderma*. Results slightly different from that observed in the decade of 2000, when the 13 patents related to research with OGM, 3 patents were linked to the fungus *Aspergillus* and 3 patents directed to the fungus *Trichoderma*.

Figure 5. Values of T(X, Z) of patents from 1975-2013

Source: Own preparation.

Regarding the pair 'content-enzyme' (Figure 5), it can be seen that there was an increase in the degree of specialization between the dimensions 'content-enzyme' until the mid-80s, followed by a significant decrease until 1990 and a new growth that lasted until 1998 (indicator peak with T(X, Z) = 1.287). From 1999, however, the mutual information indicator begins to show falling values, indicating a non-specialization between the type of research and the target enzyme. Observing information from 1999 to 2013, it appears that all kinds of research are directed to several enzymes in common. By way of example, endo-beta-1,4-glucanase is a targeted enzyme in all seven categories of research.

Despite the failure of expertise between the type of research and the enzyme target, however, looking to all the information on the screen period (1975-2013), it is possible to note a concentration of researches in general in four types of enzymes, namely, in the cellulases (endo-beta-1,4-glucanase, exo-beta-1,4-glucanase and beta-glucosidase) and endo-beta-1,4-xylanase. The interest in cellulases and xylanase makes sense if we consider that the polysaccharides present in the plant cell walls, cellulose is around 35 to 50% and that of the hemicellulose structures, xylan is one of the most representatives. In sugarcane, for example, xylan represents 50% of the hemicellulose.

Finally, with respect to the pair 'micro-organism-enzyme' (Figure 6), it can be seen that there was an increase in the degree of specialization between dimensions until 1983 (indicator peak for the whole period analyzed, with T(Y, Z) = 1.552), followed by gradual decline in mutual information indicator since 1984. The first movement, of the indicator elevation, occurs due to the presence of a small group of microorganisms directed to the synthesis of specific enzymes. A case worth mentioning is the exploration of the fungus *Trichoderma* for the synthesis of the enzyme cellulase. Incidentally, most of the patents constituent of the period of 1979-1983 involved the manipulation of species of this microorganism for cellulase synthesis.

The second movement, of gradual indicator cadence, from 1984, should be separated into two stages, due to the different triggers factors of the bearish move. The first time, verified during the second half of the 80s, is linked to the specification of cellulase enzymes (endo-beta-1,4-glucanase, exo-beta-1,4-glucanase and beta-glucosidase) in patents involving the fungus *Trichoderma*, resulting in decline in the mutual information indicator of the combination of '*Trichoderma*-cellulase'. Such combination is responsible for the high values of the indicator until 1983. The second time, verified from 1990 onwards, is linked to the expansion of the range of microorganisms subjected to the researches focused on the synthesis of enzymes. As detailed in Table 7, were found more than 80 genus of microorganisms, most of which begin to be used in the 90s, 2000 and 2010. As many of these microorganisms are directed to various enzymes in common, the mutual information indicator does not signalize a high specialization level between the dimensions of 'microorganism-enzyme'.

Figure 6. Values of T(Y,Z) of the patents from 1975-2013

Source: Own preparation.

The observation of all information from the period on screen (1975-2013), however, signals a direction of certain microorganisms for specific enzymes, in addition to important changes in the relationship between these dimensions among the decades analyzed. In the 80s, the micro-organism most mentioned was the fungus of the genus *Trichoderma*, being directed to the synthesis of the three cellulase enzymes, i.e., endo-beta-1,4-glucanase, exo-beta-1,4-glucanase and beta-glucosidase.

In the 90s, it gains importance the microorganisms of the genus *Aspergillus*, *Bacillus* and *Penicillium*. Analyzing the link between these microorganisms and enzymes, it is noted that the genus *Aspergillus* was found predominantly in the research with the enzymes endo-beta-1,4-xylanase and endo-beta-1,4-glucanase. But the genus *Bacillus* was found in patents directed to hemicellulases enzymes, particularly to the endo-beta-1,4-xylanase, and pectinase enzymes. Finally, the genus *Penicillium* showed no expertise on specific enzymes, being found in patents related to cellulases, hemicellulases and pectinases.

In the decade of 2000, the microorganisms most mentioned remained being the genus *Trichoderma*, *Bacillus*, *Aspergillus* and *Penicillium*. Two other microorganisms that become important are the genera of fungi *Chrysosporium* and *Humicola*. The analysis of the link between these micro-organisms and enzymes shows that the genus *Trichoderma* continued directed to the synthesis of the three cellulase enzymes, as evidenced during the 80s, but began to be directed also to the endo-beta-1,4-xylanase. The genus *Bacillus* also continued oriented mainly to the endo-beta-1,4-xylanase enzyme, as in the 90s, but came to be used also in studies involving the group of cellulases. The genus *Aspergillus*, in turn, was predominantly found in studies with the three cellulase enzymes and with endo-beta-1,4-xylanase, following the trend of the last decade. The genus *Penicillium*, contrary to what happened in the 90s, started to show expertise in the three cellulase enzymes. The genus *Chrysosporium* was found in patents relating to cellulases, hemicellulases and pectinases, showing no specialization in specific enzymes. Finally, the genus *Humicola* was predominantly found in researches with the enzyme exo-beta-1,4-glucanase.

In the decade of 2010, it was found the continuity in the interest of the micro-organisms cited for the previous decade, such as the genera *Trichoderma*, *Aspergillus*, *Penicillium*, *Bacillus*, *Humicola* and *Chrysosporium*. Added to this, the strengthening of the expansion phenomenon in the list of micro-organisms studied with the emergence of patents involving, notably, micro-organisms of the genus *Clostridium*, *Myceliophthora*, *Streptomyces* and *Talaromyces*s. Analyzing the link between these micro-organisms and enzymes, it is noted that, to date, the genus *Trichoderma* remains oriented to the synthesis of the three cellulase enzymes, as evidenced during the 80s, 90s and 2000, and the synthesis of endo-beta-1,4-xylanase enzymes and beta-1,4-xylosidase. The genus *Aspergillus* follows directed to the three cellulase enzymes and to the endo-beta-1,4-xylanase enzyme, reinforcing the trend of previous periods. The genus *Penicillium* is still oriented mainly to the three cellulase enzymes, as in the decade of 2000, but came to be used also in studies involving the endo-beta-1,4-xylanase enzyme. The genus *Bacillus* maintains its relationship with the group of cellulases. Since the genus *Clostridium* was found in the researches involving the three cellulase, endo-beta-1,4-xylanase and endo-beta-1,4-mannanase enzymes. The genera *Streptomyces* and *Talaromyces* showed strong links with the cellulase enzymes and endo-1,4-beta-xylanase. To complete, the genera *Chrysosporium*, *Humicola* and *Myceliophthora* did not have expertise in specific enzymes.

## **Conclusions**

The application of NK model with the information of 304 patents of the sample has allowed to verify that the search processes in 'enzymatic hydrolysis for the production of lignocellulosic bioethanol' are still in the exploratory stage. This is because, from the research of the evolution of the studied microorganisms, it is found a process of diversification of the genera of interest over the decades. Nevertheless, there are signs of paths being reinforced in some microorganisms, as can be seen in Figure 7.

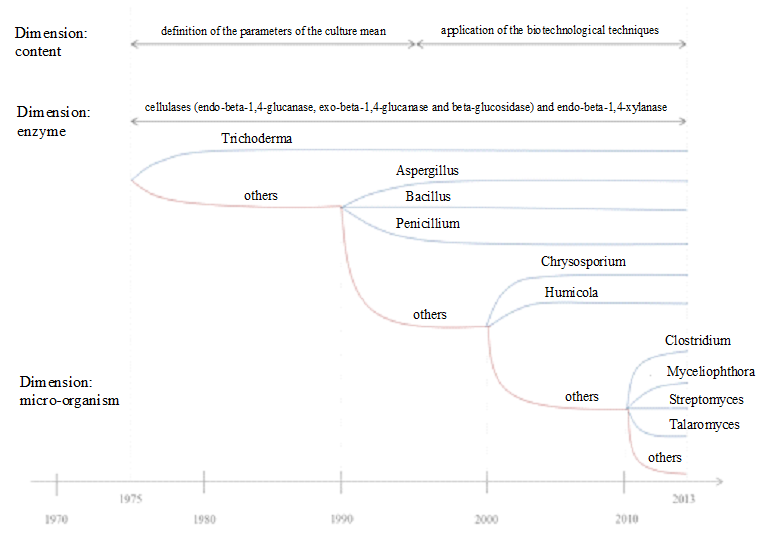


Figure 7. Technological paths in enzymatic hydrolysis for the production of second-generation bioethanol

Source: Own preparation.

The studied microorganism from the beginning of the analyzed period (1975) was the genus *Trichoderma*. In the 90s, emerge new paths with the researches being directed to the genera *Aspergillus*, *Bacillus* and *Penicillium*. In the decade of 2000, it arises the interest also in the genera *Chrysosporium* and *Humicola*. Finally, in the decade of 2010, the genera *Clostridium*, *Myceliophthora*, *Streptomyces* and *Talaromyces* start to gain ground. All these ten paths that emerged remain until the end of the period investigated.

This diversification and convergence process, in the case of microorganisms, is a typical phenomenon of biotechnology. The advent of modern biotechnology techniques (genetic engineering) is dated from the 70s. The researches concerning the enzymatic hydrolysis for the production of lignocellulosic bioethanol and patented during the 70s and 80s were based greatly on the culture of microorganisms. The researches performed from biotechnological techniques, however, only began to be patented in greater weight from 1995. Precisely the period in which it highlights the strengthening of the researches on microorganisms considered of greater potential for the synthesis of lignocellulosic enzymes.

The investigation of the evolution of the enzymes of commercial interest allowed to conclude that there is a concentration of the researches in the three cellulases and endo-beta-1,4-xylanase. That is, the most studied microorganisms are being directed to the synthesis of such enzymes. The interest in cellulases and xylanase makes sense if we consider that, from the polysaccharides present in the plant cell walls, cellulose is around 35 to 50% and that of the hemicellulose structures, xylan is one of the most representatives. In sugarcane, for example, xylan represents 50% of the hemicellulose.

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1. The second-generation bioethanol can be produced by thermochemical route, through which the lignocellulosic biomass is converted into synthesis gas (syngas), a mixture of CO, H2 and CO2 with other components in lesser quantities, which then passes through the fermentation process to give rise not only to bioethanol, as well as other products, such as n-butanol, methanol, acetic acid, etc. This route, however, is not part of the article scope (COSKATA ENERGY. Rathin Datta, Ralph Corley. **Process for fermentation of syngas from indirect gasification**. Patent number WO2011034711-A2, Available in <http://www.google.st/patents/WO2011034711A2?cl=en&hl=pt-BR>). [↑](#footnote-ref-1)
2. Currently, the comparative cost situation has become even less favorable for biofuels because of the petroleum price that has been falling since June 2014. [↑](#footnote-ref-2)
3. It is worth mentioning that, in this paper, patents information are being used to carry out this research on technological paths. And that patent is not synonymous of technological innovation. Patent involves new knowledge that may, in the schumpeterian meaning of the term, become a technological innovation in so far as there is a successful introduction of the contents of that patent on the market. However, in studies related to industrial economics and innovation, patents information is used as technological innovation *proxy*. [↑](#footnote-ref-3)