

Sustainable Industrial Development through Enzyme Technology: An approach toward cleaner production- a literature review

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Abstract: All stages of the life cycle of a product or process may adversely affect the environment by using up limited resources of materials and energy or by creating waste. Many current products and processes are now regarded as environmentally unfriendly and many are major sources of pollution. Thus, global environmental concerns will drive increased emphasis on clean industrial products and processes. Any substitution or change that reduces consumption of materials and energy and production of waste may be regarded as more environmentally friendly or “clean”. Enzyme technology may well be able to provide clean or at least less polluting alternatives to existing practices that generate environment problems over conventional process. Enzymes also contribute to safer working conditions through elimination of chemical treatments during production processes. The purpose of this article review is to summarize and discuss the findings of these studies and to recommend further developments regarding environmental assessment and implementation of the technology. The study provide an overview of current and future characteristics of global market for industrial enzyme.

Keywords: Clean technology, Global Market, Enviromental pollution, Life Cycle Assesment, Bio based product, Government bureauracy

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I. Introduction

In a world with a rapidly increasing population and approaching exhaustion of many natural resources, enzyme technology offers a great potential for many industries to help meet the challenges they will face in years to come. Industries around the world are looking for alternative technologies that can deliver the increasing numbers of products that are in demand every year while consuming fewer resources and having a lesser impact on the environment. Enzyme technology is a promising means of moving toward cleaner industrial production over the conventional chemical processes. The introduction of enzymes as effective catalysts working under mild conditions results in significant savings in resources such as energy and water for the benefit of both the industry and the environment. Compared with conventional chemical catalysts, enzyme catalysis is highly specific and it functions under temperatures, pressures and pHs that are compatible with life. Unlike many processes of conventional synthetic chemistry, enzymes require nontoxic and noncorrosive conditions (Maria Gavrilesqua and Yusuf Chisti, 2005). Implementing enzymatic processes in place of conventional processes generally results in a reduced contribution to global warming and also a reduced contribution to acidification, eutrophication, photochemical ozone formation and energy use (Kirk-Othmer, 2005). Enzymes are readily biodegradable and usually lead to reduced or no toxicity when they reach the environment after use in industrial production (Soetaert and Vandamme, 2010).

Enzymes are mainly proteins produced by all living organisms; they accelerate or catalyse numerous biochemical reactions. Enzymes found in nature have been used since ancient times in the production of food products, such as cheese, sourdough, beer, wine and vinegar, and in the manufacture of commodities such as leather, indigo and linen (Ole Kirk, et. al. 2002). The use of enzymes to produce goods for human consumption dates back at least 2000 years, when microorganisms were used in processes such as leavening bread and saccharification of rice in koji production (Demain and Fang, 2000). The mechanism of the enzymes was unknown until 1877, when Moritz Traube proposed that “protein-like materials catalyze fermentation and other chemical reactions ...” Later, the historic demonstration by Buchner in 1897, showing that alcoholic fermentation could be carried out using cell-free yeast extract, appears to be the first application of biocatalysis. The word ‘zymase’ was coined to describe this cellfree extract (Bornscheuer and Buchholz, 2005), which was

the initial recognition of what is now called an 'enzyme'. There are currently around 5500 known enzymes (BRENDA, 2012), classified based on the type of reaction they catalyze (oxidoreductases, transferases, hydrolases, lyases, Isomerases, ligases).

Enzymes for industrial use are produced by growing bacteria and fungi in submerged or solid state fermentation. With submerged being the primary fermentation mode, the unit operations in enzyme production involve fermentation followed by cell disruption and filtration. The crude enzyme is further purified by precipitation followed by centrifugation and vacuum drying or lyophilization, collectively known as "downstream processing" (Kim et al., 2009). The development of fermentation processes during the later part of the last century, aimed specifically at the production of enzymes by use of selected production strains, made it possible to manufacture enzymes as purified, well-characterized preparations even on a large scale. This development allowed the introduction of enzymes into true industrial products and processes, for example, within the detergent, textile and starch industries (Ole Kirk, et. al. 2002). Industrially produced enzymes are used in a broad variety of production processes, such as pulp and paper production, leather production, textile production, detergent production, food production, beverage production, animal feed production, pharmaceuticals production, fine chemicals production, cosmetics production and biodiesel production (Kenthorai Raman Jegannathan, Per Henning Nielsen; 2012).

The purpose of the present review is therefore: 1) to provide an overview of Life Cycle Assessment (LCA) and Environmental Impact Assessment (EIA) studies reported so far comparing enzymatic processes with conventional processes; 2) to summarize the main results of the studies; 3) to draw the general conclusions on whether and to what extent enzymatic or enzyme-assisted processes are environmentally favourable as alternatives to conventional technology; 4) to recommend further development of environmental assessment of enzymatic processes and implementation of enzyme technology in industry; 5) to provide an overview of current and future characteristics of global market for industrial enzyme.

Enzymatic process: The cleaner production technique

Clean technology: a conceptual and procedural approach to industrial activities that demands that all phases of the life cycle of a product or of a process should be addressed with the objective of prevention or minimisation of short- and long-term risks to human health and to the environment (OECD, 1998). The essential feature of clean technology is avoidance of environmental damage at the source. There are three main drivers of clean technology: economic competitiveness, with companies considering the advantages of clean products and processes in terms of market niches or cost advantages; government policies, which enforce or encourage changes in manufacturing practices; and public pressure, which takes on strategic importance as companies seek to establish environmental legitimacy (OECD, 1998). The paradigm shift that has taken place since the early 1990s: the emphasis is no longer on the removal of pollutants from an already damaged environment, but on the need to reshape industrial process technologies to prevent pollution at the source (OECD, 1998).

Enzyme technology is a powerful enabling technology for achieving clean industrial products and processes that can provide a basis for industrial sustainability. Sustainable industrial development means continuous innovation, improvement, and use of "clean" technologies to make a fundamental change in pollution levels and resource consumption. An environmentally friendly process would, in principle, have: a) low consumption of energy and non-renewable raw materials (especially fossil fuel feedstock) relative to the products or services delivered; and b) reduction or elimination of waste (including materials and energy recycling and energy use) (OECD, 1998).

Measurement of Clean Industrial Production process:

Measuring the cleanliness of an industrial product or process is essential but complex; Life Cycle Assessment (LCA) is the best current tool for making this determination while use of the 'carbon footprint' concept and Environmental Impact Assessment (EIA) is limited to a few studies. LCA consists of four steps: definition of aims and scope, inventory analysis (collection of the relevant inputs and outputs), impact assessment (including "weighting", which is the most disputed aspect of LCA because it requires judging the relative importance of different factors, e.g. CO₂ emissions versus mercury pollution of soil), and fourth, interpretation. There is a high degree of consensus on the methodological framework, although the task of collecting the data can be very onerous (OECD, 1998). In 1993, Life Cycle Assessment (LCA) was defined by the Society of Environmental Toxicology and Chemistry (SETAC) as: "... a process used to evaluate the environmental burdens associated with a product, process or activity by identifying and quantifying energy conversion, materials used, and waste released to the environment, to assess the impacts of those energy and materials uses and releases to the environment, and to identify and evaluate opportunities to obtain environmental developments" (OECD, 1998). Almost no LCA studies were carried out before the beginning of the 1980s, but their numbers increased from the mid-1980s and gained momentum in the 1990s. In Germany, there have been at least 16 studies a year since 1990; the figure of seven studies for 1996 should by no means be

interpreted as a downward trend, since at the time of the statistical survey (summer 1996) a considerable number of studies had not been completed. Moreover, there is always a time lag between completion of a study and its public disclosure. In Switzerland the number of LCA studies has stood at 15-20 a year during the present decade; and in Sweden the boom began in about 1992, since when almost the same number of studies has been recorded as for Germany (OECD, 1995). . A number of comparative environmental assessment studies have been conducted in the past 15 years to investigate whether these properties of enzymatic processes lead to environmental improvements and assess whether they could play a role in moving toward cleaner industrial production.

Potential use of different enzymes in industrial processes:

The majority of currently used industrial enzymes are hydrolytic in action, being used for the degradation of various natural substances. Proteases remain the dominant enzyme type, because of their extensive use in the detergent and dairy industries. Various carbohydrases, primarily amylases and cellulases, used in industries such as the starch, textile, detergent and baking industries, represent the second largest group (Godfrey T, West SI, 1996). Certain sectors have been clearly identified as areas for cleaner practices.

Table: Technical issues affecting the wider industrial application of biotechnology

Industrial sector	Technical issues	Potential developments/solutions	Class of enzyme used		
Chemicals					
- Commodities	Reaction conditions (temperature, solvents)	Biodiversity (search and discovery) Protein engineering	Lipase, Acylase, Lipolase, Penicillin amidase, Nitrilase, Protease, Amylase		
- Plastics, polymers	Persistence	Biodegradable biopolymers			
	Functionality	Enzyme synthesis/modification			
- Pharmaceuticals	Discovery rate	Bioinformatics			
	Manufacturing costs	Biodiversity/targeted screening			
		Combinatorial chemistry/biochemistry			
		Lead compounds based on non-natural substances			
Pulp and paper					
	Lignin removal	Biobleaching Transgenic trees	Lipase, Protease, Amylase, Ligninase, Xylanase, Cellulase		
	Waste recycling	Recycling of pulp "fines"			
	Chemical modification	By-product removal			
Textiles and leather					
	Chemical texturing and dyeing	Enzymes	Cellulase, Pectate lyase, Catalase, Laccase, Protease, Amylase, Lipase,		
	Acid and alkaline processing conditions	Extremophiles			
Food and feed					
	Maintenance of sterile conditions	Thermophilic organisms	Amylase, Pectinase, Lipase, Protease, Xylanases, Phytase, Lactase, Glucose oxidase, Lipooxygenase		
		Extremozymes			
Metals and minerals					
	Metal toxicity	Biodiversity	Glutathione peroxidase, Metalloprotease, Collagenase		
	Processing rates	Genetic manipulation			
	Waste management				
Energy					
	Low grade fossil fuels	Biodesulphurisation	Xylanases, Cellulase, Pullulanase, Glucose isomerase		
	Recovery	Enhanced oil recovery			
	Sustainability	Hydrogen manufacture			
	Land requirement for biofuel feedstocks				

Supporting case studies showing pollutant reduction at source and acceptability of enzymatic process world over

The OECD Task Force on Biotechnology for Sustainable Industrial Development has recently published a report entitled “The Application of Biotechnology to Industrial Sustainability”. This report provides case studies of how companies in a wide range of industrial sectors have used biotechnology to reduce the cost and environmental impact of their production activities. Summaries of the case studies are provided below. The six sectors examined are responsible for a large share of the industrially generated pollution in OECD countries: chemicals, pulp and paper, textiles and leather, food and feed processing, metals and minerals, and energy (OECD, 1998).

Enzymes usually function in an aqueous solution and this can reduce the requirement in equivalent conventional chemical processes for organic solvents that will later need to be recycled or disposed of by incineration. **Biochemie** (Germany/Austria), a subsidiary of Novartis, has developed an enzyme-catalysed process for manufacture of the antibiotic cephalosporin. The efficiency of the enzymes was optimised by genetically modifying the micro-organisms that produce the enzymes. When compared to the conventional chemical process, the enzymatic process produces 100 times less waste solvent to be incinerated and, as a result, the cost of production and the potential environmental impact of the process are both reduced.

Mitsubishi Rayon Company (Japan) produces acrylamide, a chemical used to produce acrylic polymers. The conventional chemical process for producing acrylamide from acrylonitrile involves high temperature and the use of either a copper catalyst or sulphuric acid. Mitsubishi Rayon has developed a bioprocess which instead uses a naturally occurring enzyme, nitrile hydratase, to catalyse the conversion of acrylonitrile into acrylamide. The performance and yield of this enzyme has been optimised by genetically engineering the micro-organism which naturally produces the enzyme. The enzyme-catalysed process uses 80% less energy, saves costs and yields higher purity acrylamide than the conventional chemical process.

The conventional chemical process for producing certain polyesters involves the use of either a titanium or tin-based catalyst with solvents and inorganic acid at high temperature (200 degree C). **Baxenden Chemicals** (United Kingdom) has developed a bioprocess that uses the enzyme lipase from the yeast *Candida antarctica* to catalyse the polymerisation reaction at a much lower temperature (60 degree C). The lipase gene was transferred into a genetically engineered industrial strain of *E. coli* bacterium to reduce the cost of producing the enzyme. The enzyme-catalysed polymerisation process, when compared with the conventional process, eliminates the use of organic solvents and inorganic acids and yields energy savings of about 2000 megawatts annually at full industrial scale operation. The polymer from the bioprocess also has a more uniform polymer chain length. This results in a melting point over a narrower range of temperature than the conventional polyester, making it more valuable for use as a hot-melt adhesive. Thus, there were both environmental and economic benefits from implementing the enzyme-based bioprocess.

Often, food processing uses large quantities of water and produces large quantities of organic waste. Biotechnology can help reduce water usage as well as the production of organic waste. **Cereol** (Germany) has implemented an enzyme-based system for the degumming of vegetable oil during purification after extraction. This bioprocess was compared with the conventional degumming process that used sulphuric acid, phosphoric acid, caustic soda and large quantities of water. The enzyme system eliminated the need for treatment with strong acid and base, reduced water use by 92% and waste sludge by 88% and resulted in an overall cost reduction of 43%.

Domtar (Canada) has begun to use the enzyme xylanase, supplied by Iogen Corporation (Canada) as an auxiliary brightening agent (this process is called “bio-bleaching”) for wood pulp in paper making. The enzyme opens up the lignin structure of the wood pulp so that it takes 10-15% less chlorine dioxide to achieve the desired level of brightness. Iogen has reduced the production cost and improved the performance of xylanase by genetically engineering the fungus from which it is extracted. The use of xylanase has helped Domtar reduce the amount of organically bound chlorine in waste water by 60% and the cost of bleaching chemicals by 10-15%.

Budel Zinc (Netherlands) is a major producer of zinc. The acidic waste water from its zinc refinery contains zinc and other metals (tin, copper, nickel, manganese, chromium, lead and iron). The conventional process for treating this waste water involves neutralising it with lime or limestone, which results in large quantities of gypsum contaminated with heavy metals. Budel has developed a bioprocess that uses sulphate-reducing bacteria to capture and recycle zinc and other metals in its waste water as metal sulphide precipitate. The metal sulphide precipitate is recycled back into the refinery feedstock. This process has resulted in a 10 to 40-fold decrease in the concentration of heavy metals in the refinery wastewater and eliminated the production of metal-contaminated gypsum which is a hazardous solid waste by-product.

Ethanol is one renewable fuel whose production is increasing rapidly in response to the need for transportation fuels that produce lower net emissions of greenhouse gases (GHG). Ethanol is produced by fermentation of sugars (such as glucose) using brewers’ yeast. The sugar can come from cornstarch. It takes

considerable energy to produce corn, however, so the net reduction in GHG emissions is around 40-50% when ethanol from corn is used to replace gasoline (petrol). If wood cellulose and waste materials are used as the source of sugar to produce ethanol, the net reduction in GHG emissions is larger, around 60-70%. Therefore cellulose-containing materials are, from a GHG perspective, the material of choice for producing ethanol. However, the lignin in woody plant material can prevent full conversion of cellulose into fermentable sugar. **Iogen Corporation** (Canada) has developed a process utilising cellulase enzymes that maximise the conversion of cellulose into fermentable sugar. The yield and activity of the cellulose enzymes has been optimised using biotechnology. **Iogen** is in the scale-up phase of the technology and indications are that the cost of ethanol produced in this manner will be competitive with the cost of gasoline produced from oil costing USD 25 per barrel (OECD, 2001).

Global Market for Enzyme in Industrial Application

A recent report regarding the global market for industrial enzyme published by BBC Research states that the estimated value of worldwide use of industrial enzyme has grown from \$ 1 billion in 1995 to \$ 7.1 billion by 2018, registering a five year compound and growth rate (CAGR) of 8.2%. North America is expected to account for the largest share of the molecular biology enzymes and kits & reagents market in 2016. The Asia-Pacific region is expected to grow at the highest CAGR during the forecast period. The high growth of this region can be attributed to increasing government interest in biomedical and biotechnology industries in China, presence of bioclusters in China, outsourcing of clinical research activities in China, conferences & meetings in Japan. Growing pharmaceutical industry backed by government initiatives in India, development of bioclusters in India, Korea-U.S. free trade agreement, and initiatives by non-profit organizations in Asia-Pacific. According to Global Industry Analysis-The Biotechnology industry in India is expected to reach US\$11.6 billion by 2017 (FICCI, 2005). The year-on-year growth of the biotech market is expected to accelerate driven by high demand for vaccines, biopesticides, biofertilizers, biodiesel, biotherapeutics and medical devices in India as well as at the global level.

India is already ranked among the top 12 biotech destinations worldwide and third largest in the Asia-Pacific region. The field of Biotechnology in the last decade has already made a significant impact in agricultural, industrial, pharmaceutical and medical sectors (FICCI, 2005). The enzymes sector in India has been growing at about 11.47 percent in the last 10 years and is expected to see a growth of 11.17 percent in 2012-13. In recent years, enzymes have also found numerous applications in the food, pharmaceutical, diagnostic and chemical processing industries. The Indian market is spearheaded by global leaders such as Novozymes South Asia, Danisco (India), DSM Nutritional Products India, and Chr. Hansen (India). Besides, there are many domestic enzyme manufacturers, such as Advanced Enzymes, Lumis, Maps and Anthem (FICCI, 2005). The pharmaceutical enzymes segment is relatively nascent and is the focus of a small group of specialized manufacturers. Meanwhile, the textile and leather enzyme segments are mature, while the detergent enzymes segment is in the growth stage (FICCI, 2005). The industry is concentrated around major Bioclusters: Maharashtra, Karnataka, Uttar Pradesh, Andhra Pradesh, NCR & Gujarat. Bengaluru is the home to the biggest bio cluster in India with 137 Biotechnology companies, making it 40% of the total 340 such units in the country as per Government of India-Ministry of MSME report August 2012 (FICCI, 2005).

National and International Policies to Promote Bio – Based Product

A few examples of specific policies that have been developed to promote the use of biobased products include: In Europe, the European Commission is developing a demand-based innovation policy for biobased products. The aim of the so-called Lead Market Initiative (known as LMI) for biobased products is to promote and stimulate innovation by strengthening the demand base. The added value of the LMI is about developing a prospective, concerted and tailored approach of regulatory and other policy instruments, including legislation, public procurement, standardisation, labelling, certification, and complementary instruments. Although an advisory group has developed a series of recommendations to stimulate market uptake and development, these measures still have to be implemented. Other national demand-driven policies often focus on the sustainability agenda (including green public procurement) and are often implemented as a mix of public procurement procedures, legislation and direct financial incentives. In the United States, the BioPreferred programme aims to increase the purchase and use of renewable, environmentally friendly biobased products. In Japan, in 2002 the government initiated the Biomass Nippon Strategy, requiring that 20% of all plastics consumed in the country be sourced renewably by 2020. This prompted Toyota, NEC and others to accelerate levels of R&D into biobased plastics and to raise the biobased content of their products. Biobased chemicals and bioplastics benefit from usage, waste management, and labelling legislation. In China, support for biobased chemicals includes numerous incentives for producers and a preferential tax treatment for selected firms in emerging biochemical industries. In addition, since 2005, a specific programme promotes production and consumption of biodegradable plastics. In Korea, the government supports the use of biodegradable materials in refuse bags and

fishing nets. One-time use products cannot be made from conventional plastics, and polystyrene is banned in food packaging. (OECD, 2011). In India, the government has introduced a number of important legislations in order to support and promote the growth of biotechnology industry in the country. Government has proposed the establishment of the National Biotechnology Regulatory Authority (NBRA) to provide a consistent mechanism for regularity approval.

II. Suggestions/ Publication Analysis

As less than 1 per cent of the micro-organisms in nature have been cultured, the search is on to examine the remaining, unexplored microbial biodiversity which presumably holds a great wealth of biocatalytic potential (OECD, 1998). There is a need to communicate to the public and to their government representatives the value of clean technology and the role of biotechnology for achieving industrial sustainability. Traditional thinking among manufacturers and suppliers, lack of knowledge and governmental bureaucracy during approval of new solutions in many countries tend to delay broader implementation of enzymatic processes in industry. Industry, government and the public are the main stakeholders for the development of environmentally friendly products and processes. Enzyme technology continues to develop new, precise tools to improve a wide range of production processes (e.g., for food, microbial, chemical, agricultural, applications.). These tools offer a broad array of benefits including reduced energy and raw material consumption. The savings includes less waste and fewer environmental pollutants. The enzyme industry seeks to educate consumers about the importance of enzymes in everyday life, in the higher education sector, the main need is to broaden the training of biotechnologists and engineers and to integrate LCA, sustainability and other relevant concepts into curricula and hence future views. Similar shifts in perspective are required in the education of non-scientific industrial staff (retraining). Finally, there is a clear need to enhance the scientific awareness of government regulators and their constituencies on issues of biotechnology for clean industrial products and processes.

III. Conclusion And Future Perspective

Enzymes can often replace chemicals or processes that present safety or environmental issues. For example, enzymes can: Replace acids in the starch processing industry and alkalis or oxidizing agents in fabric desizing, Reduce the use of sulfide in tanneries, Replace pumice stones for “stonewashing” jeans, Allow for more complete digestion of animal feed leading to less animal waste, and Remove stains from fabrics. Clothes can be washed at lower temperatures, thus saving energy. Enzymes can be used instead of chlorine bleach for removing stains on cloth. The use of enzymes also allows the level of surfactants to be reduced and permits the cleaning of clothes in the absence of phosphates. Waste generation and disposal is another area that has received significant attention. The focus has been on reduction of waste generation through both voluntary and regulatory initiatives. This is evident in initiatives to increase the use of recyclable materials in product development and in the increased use of renewable materials in products.

The purpose of moving towards cleaner industrial products and processes is to achieve specific societal, economic and safety objectives. Moreover, specific toxic materials are identified and targeted for reduction or elimination. The objective is to change the industrial processes that rely on these chemicals. The alternatives can then be specified. Both internationally and nationally, specific chemicals have been identified for reduction or elimination. In general, these are persistent, bioaccumulative, and toxic; in particular, the highly chlorinated chemicals have been targeted for reduction or elimination. For reliable hazard identification of environmental contaminants/pollutants, knowledge of the effects on different levels of biological organisation is necessary.

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