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Review

Environmental assessment of enzyme use in industrial production – a literature review

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ABSTRACT

Enzymatic processes have been implemented in a broad range of industries in recent decades because they are specific, fast in action and often save raw materials, energy, chemicals and/or water compared to conventional processes. 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. The purpose of this review is to summarize and discuss the findings of these studies and to recommend further developments regarding environmental assessment and implementation of the technology. Life Cycle Assessment (LCA) has been widely used as an assessment tool, while use of the 'carbon footprint' concept and Environmental Impact Assessment (EIA) is limited to a few studies. Many studies have addressed global warming as an indicator and several studies have furthermore addressed other impact categories (acidification, eutrophication, photochemical ozone formation, energy and land use). The results show that 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 to the extent that this has been investigated. Agricultural land has been addressed in few studies and land use savings appear to occur in industries where enzymatic processes save agricultural raw materials, whereas it becomes a trade-off in processes where only fossil fuels and/or inorganic chemicals are saved. Agricultural land use appears to be justified by other considerable environmental improvements in the latter cases, and the results of this review support the hypothesis that enzyme technology is a promising means of moving toward cleaner industrial production. LCA gives a more complete picture of the environmental properties of the processes considered than EIA and carbon footprint studies, and it is recommended that researchers move toward LCA in future studies. Tradition, lack of knowledge and bureaucracy are barriers to implementation of enzymatic processes in industry. Education and streamlining of public approval processes etc. are means of overcoming the barriers and accelerating the harvesting of the environmental benefits.

1. Introduction

The production of daily life products such as paper, textile, food, feed, chemicals and pharmaceuticals consumes large amounts of raw materials and energy, and generates large amounts of waste with an adverse impact on our environment and quality of life (OECD, 2009; European Commission, 2009). The growing global population and improving economies in many countries increase global consumption and thereby the pressure on environment (UNFPA, 2008; UNEP, 2011a) and it is well recognized that there is an urgent need to reduce the impact per produced unit of product to sustain human needs without compromising the natural resource basis (UNEP, 2011b). Industries around the world are thus 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.

The use of bio-based materials and nature's production processes, known as industrial biotechnology (Kirk et al., 2002; Soetaert and Vandamme, 2010), is one such alternative technology

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which could be used to either replace or supplement conventional technologies in moving toward cleaner production processes (Kirk et al., 2002; Bornscheuer and Buchholz, 2005; OECD, 2009; Haas et al., 2009; Wohlgemuth, 2009).

Among biotechnologies, enzymatic processing is seen as one of the promising and sustainable alternatives to conventional processing (IPTS, 1998; Vigsoe et al., 2002; Thomas et al., 2002; Kirk-Othmer, 2005). Enzymes are proteins produced by all living organisms; they act as a catalyst for numerous biochemical reactions. Apart from being catalysts in vivo, enzymes can also be catalysts in vitro for various reactions, including in industry.

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 cell-free extract (Bornscheuer and Buchholz, 2005; Soetaert and Vandamme, 2010), 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, and ligases). Specific enzyme names refer to the substance on which they act. An enzyme that acts on cellulose, for example, is known as cellulase, and an enzyme that acts on protein is named protease, etc. (IUB, 1961).

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; Soetaert and Vandamme, 2010).

Enzymes are highly specific and they usually act under milder reaction conditions than traditional chemicals. Furthermore, they are readily biodegradable and usually lead to reduced or no toxicity when they reach the environment after use in industrial production (Kirk-Othmer, 2005; Soetaert and Vandamme, 2010). These properties allow manufacturers to produce the same or sometimes even better quality products with less raw material, chemical, water and/or energy consumption and with less problematic waste generation than traditional processes (Thomas et al., 2002; Soetaert and Vandamme, 2010). Industrially produced enzymes are used in a broad variety of production processes, such as pulp and paper production (Jiménez et al., 1999; Nguyen et al., 2008), leather production (Dayanandan et al., 2003; Saravanabhavan et al., 2004; Valeika et al., 2009; Kandasamy et al., 2012), textile production (Aly et al., 2004; Vankar et al., 2007; Chen et al., 2007; Zhou et al., 2008), detergent production (Hemachander and Puvanakrishnan, 2000; Saeki et al., 2007), food production (Minussi et al., 2002; Ramos and Malcata, 2011), beverage production (Grassin and Fauquembergue, 1996; Okamura-Matsui et al., 2003), animal feed production (Gado et al., 2009; Zhu et al., 2011), pharmaceuticals production (Bonrath et al., 2002; Woodley, 2008), fine chemicals production (Panke et al., 2004; Gavrilescu and Chisti, 2005), cosmetics production (Sim et al., 2000; Lods et al., 2001) and biodiesel production (Kumari et al., 2007; Hernández-Martín and Otero, 2008).

The environmental benefits of enzymatic processes over conventional processes in various industries have been discussed in several books, articles and reports over the past decade (Falch, 1991; Sime, 1999; Wandrey et al., 2000; Zaks, 2001; Kirk et al., 2002; Sijbesma, 2003; Olsen, 2008; Kirk-Othmer, 2005; Gavrilescu and Chisti, 2005; Herbots et al., 2008; Haas et al., 2009; OECD, 2009; Kanth et al., 2009; Soetaert and Vandamme, 2010; Mahmoodi et al., 2010). All agree that enzymatic processes are favorable to the environment compared with the traditional processes. However, these are only based on qualitative judgments, and a concrete justification is needed as it cannot be excluded that the production of enzymes (Nielsen et al., 2007; Kim et al., 2009) and any helping agents for the enzymatic processes requires more energy and raw materials than it saves. Quantitative environmental impact assessments are therefore necessary in order to assess the actual environmental benefits of enzymatic processing.

LCA and EIA are versatile tools for quantitatively assessing the environmental impacts of products and systems (Wenzel et al., 1997; Guinée, 2002; ILCD, 2010). Comparative LCA and EIA of enzymatic processes versus conventional processes began in the late 1990s (OECD, 1998). It became increasingly used during the first decade of this century (Kallioinen et al., 2003; Fu et al., 2005; Nielsen and Wenzel, 2006), when concepts, databases, tools and standardizations were sufficiently developed, and have been used extensively since then. Results are published in many different reports and journals in various fields and it has long been difficult to gather all the information and draw the first more general conclusions on enzymatic processes as a means of achieving cleaner industrial production. The purpose of the present review is therefore: 1) to provide an overview of LCA and EIA studies reported so far comparing enzymatic processes with conventional processes; 2) to summarize the main results of the studies; 3) to draw the first more general conclusions on whether and to what extent enzymatic or enzyme-assisted processes are environmentally favorable as alternatives to conventional technology; and 4) to recommend further development of environmental assessment of enzymatic processes and implementation of enzyme technology in industry.

2. Methods and scope

This study focuses on industrial processing and addresses cases ranging from lab-scale to full-scale production where conventional production technology is partially or fully replaced with enzyme-assisted production technology (Fig. 1A) by means of industrially produced enzymes.

Replacement of conventional materials with bio-based materials is outside the scope of the study, even if one or more enzymatic processes may have been involved (Fig. 1B). The reason is that a review of environmental assessments of bio-based materials is a comprehensive subject in itself (González-García et al., 2011; Álvarez-Chávez et al., 2012; Weiss et al., 2012) and we find it meaningful to distinguish between the material-oriented studies of biomaterials and the process-oriented studies of enzyme technology.

The review is based on literature from the entire world, and since the subject is still in development we have included not only comparative LCA and EIA studies reported in peer-reviewed journals but also studies reported in technical journals, books, conference proceedings and publically available reports.

Studies published in technical journals and books, etc. are often summaries of comprehensive background reports with third-party external review according to standards such as ISO 14040. Use of standards and reviews is important when evaluating the credibility of the results, and the name of the standard and type of review has been investigated (in some cases by contacting authors) and reported in a summary table (Table 1).

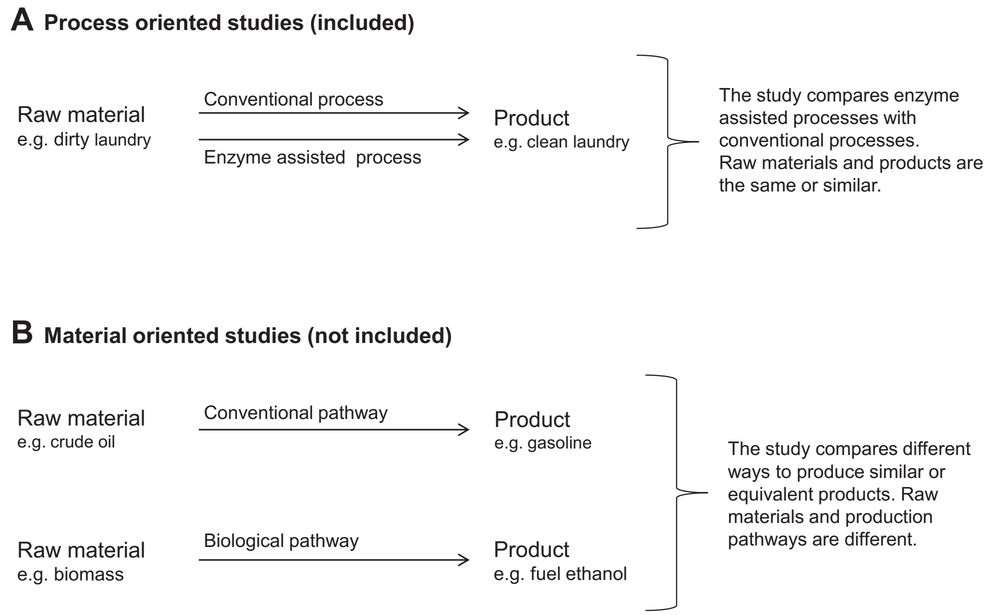

Fig. 1. Principle difference between process and material oriented studies of biotechnological solutions. Process oriented studies of conventional versus enzyme-assisted technology are included in this review. Material oriented studies are not included even though enzymes may have played a role in the biological pathway.

Based on the above criteria, 28 comparative LCA or EIA studies have been identified and organized with reference to i) industry segment and ii) enzyme type applied. Processes and effects of enzymes are explained briefly and effects on climate change have been tabulated for each industry segment (see Table 1). Climate change has been selected as the primary impact indicator because it is the subject of much attention at the moment and because we find that it is the best single indicator for a broad range of effects in the present context. We acknowledge that a range of effects are overlooked when only one indicator is addressed in the summary table and refer to the background literature for further details.

Finally, it is worth noting that all the comparative LCA and EIA studies reported in this paper refer to specific conditions in specific production plants. One should therefore be careful in generalizing at the detailed level as there may be considerable variation from factory to factory and from country to country, etc.

In the following sections, the role of enzymes in a broad range of industries is described in brief along with their environmental impact.

3. Pulp and paper industry

Paper and paperboard can be produced from either a virgin resource of wood or from recycled material. The traditional pulp and paper production process is based on chemicals and mechanical processing, which consumes large amounts of raw materials and energy and creates considerable pressure on the environment (Bajpai, 2005; Rosenfeld and Feng, 2011). A range of enzyme applications in the pulp and paper industry which have been the subject of LCA studies are described in the following sections.

3.1. Cellulase

3.1.1. Refining

Conventional thermomechanical pulping is an energy-intensive process in which two grooved metal plates are used to refine wood into pulp (Kallioinen et al., 2003). Energy consumption for refining can be reduced, however, by softening the wood fibers with a cellulase enzyme prior to processing (Pere et al., 2000).

3.1.2. Deinking

The pulp from recycled paper contains ink that needs to be removed before the paper can be used again. The conventional deinking process requires large amounts of chemicals such as NaOH, $NaHSO_3$ and H_2O_2. However, use of cellulase enzymes in the deinking process softens the recycled pulp and facilitates the release of the ink. This saves on processing time and deinking chemicals (Patrick, 2004).

Skals et al. (2008) used LCA to compare the environmental impact of cellulase-assisted processing and conventional processing in refining and deinking. They found that the energy and chemical savings achieved led to a net reduction in fossil fuel consumption and thereby reduced emissions of energy-related atmospheric pollutants such as CO_2 (which contributes to global warming), NO_x (which contributes to acidification and nutrient enrichment), SO_x (which contributes to acidification) and volatile organic compounds (which contribute to photochemical smog formation). Use of agricultural land came out as the single trade-off. The reason was that enzyme production consumes carbohydrates for fermentation (see Introduction) and therefore uses agricultural land. This is a common issue in enzyme-assisted processes, which save energy and/or inorganic chemicals but no agricultural products, and is discussed in Section 13. The details of the comparative LCAs on cellulase use in pulp production are summarized in Table 1.

3.2. Laccase

The main constituents of wood are cellulose, lignin and xylan. In paper-making, lignin is the substance that gives a dark color to the pulp and it needs to be removed to make bright paper qualities. In traditional chemical pulping, lignin is removed by adding large amounts of chlorine and alkali chemicals in a process called ‘bleaching’. Alternatively, a lignin-degrading enzyme (laccase) can be used in the bleaching process. Fu et al. (2005) conducted a preliminary LCA to compare the laccase-assisted bleaching and

Table 1
Summary of comparative environmental assessments of enzymatic and conventional processes in various industries. Greenhouse gas (GHG) savings reported in the table refer to the specific production conditions addressed in the actual studies and specific figures cannot necessarily be generalized.

Industry	Application process	Enzyme	Function of enzyme	Savings obtained by enzyme use	Functional unit, FU	GHG saving, kg CO_2 equivalents/FU (unless otherwise noted)	Impact assessment method	Scale of production	Reference, publication type
Pulp and paper	Thermo-mechanical pulping	Cellulase	Softens wood chips	Energy	1 ton pulp	145	EI'95[a]	Full-scale	(1), peer reviewed journal paper
						25	IPCC[b]	Full-scale	(2), report
	Deinking	Cellulase	Acts on recycled fibers and facilitates ink loosening	Chemicals	1 ton recycled pulp	5	EI'95	Full-scale	(1), peer reviewed journal paper
	Bleaching	Laccase	Oxidizes lignin and enhances lignin removal	Bleaching chemicals, energy	1 ton bleached pulp	−9%	EI'95	Full-scale	(3), peer reviewed journal paper
		Xylanase	Hydrolyzes xylan and enhances lignin extraction	Bleaching chemicals	1 ton virgin pulp	37	EI'95	Lab-scale	(1), peer reviewed journal paper
	Pitch control	Lipase	Hydrolyzes pitch	Cleaning agent, talc, energy	1 ton virgin paper	9	EI'95	Full-scale	(1), peer reviewed journal paper
	Stickies control	Esterase	Hydrolyzes glue and controls stickies	Talc, solvent, energy	1 ton recycled paper	13	EI'95	Full-scale	(1), peer reviewed journal paper
Leather	Beam house	Protease, lipase	Facilitates hair and fat removal from hides	Chemicals, energy	1 ton hide	97	EI'95	Full-scale	(4), technical journal paper[c]
Textile	Scouring	Pectate lyase	Degrades pectin and assist in removal of wax etc. from raw cotton	Energy, water, chemicals, cotton	1 ton yarn	990	EI'95	Full-scale	(5), chapter in a book[c]
	Bleaching	Catalase	Hydrolyzes hydrogen peroxide to oxygen and water	Heat, electricity, water	1 ton yarn	410	EI'95	Full-scale	(5), chapter in a book[c]
	Bleaching	Arylesterase	Perhydrolyzes to form peracetic acid (bleaching activator)	Chemical, cotton, energy	1 kg cotton fabric	1.3	IMPACT 2002+	Full-scale	(6), conference poster[c]
Detergent	Laundry washing	Protease, lipase, amylase, cellulase	Removes stains from laundry	Chemicals, energy	3 kg laundry	0.3	EI'95	Model detergent	(7), technical journal paper[c]
Food and beverage	Degumming of soybean oil	Phospo-lipase	Hydrolyzes phospholipids	Oil, chemicals	1 ton oil	45	EI'95	Full-scale	(8), technical journal paper[c]
	Hard stock production	Lipase	Interesterification of vegetable oil	Chemicals, energy	1 ton hard stock	340	EI'95	Lab/full-scale	(9), peer reviewed journal paper
	Fruit juice production	Pectinase	Breaks down pectin in fruit	Fruits	1 L juice	CO_2 emission reduced	IPCC EI'99	Full-scale	(10), annual report
	White bread production	Amylase	Degrades starch and delays hardening of bread	Bread	100 breads sold	5.4	EI'95	Full-scale	(11), technical journal paper[c]
	Steamed bread production	Amylase, lipase			1 ton bread sold	39	CML 2 baseline 2000		(12), report[c]
	Mozzarella cheese production	Phospho-lipase	Hydrolyzes phospholipids in milk	Milk	1 ton cheese	220	EI'95	Full-scale	(13), peer reviewed journal paper[c]
	100% Barley beer production	Amylase, protease etc.	Converts starch to fermentable sugars	Barley, energy	100 L beer	2.5	CML 2 baseline 2000	Full-scale	(14), technical journal paper[c]
		Amylase, protease		Energy		4.7	EI'99		(15), conf. presentation

(continued on next page)

Table 1 (*continued*)

Industry	Application process	Enzyme	Function of enzyme	Savings obtained by enzyme use	Functional unit, FU	GHG saving, kg CO_2 equivalents/FU (unless otherwise noted)	Impact assessment method	Scale of production	Reference, publication type
Animal feed	Pig feed production	Xylanase	Depolymerizes xylans and enables better digestion	Feed	1 ton pig feed	78	EI'95	Full-scale	(16), peer reviewed journal paper
	Pig feed production	Phytase	Hydrolyzes phytate and releases phosphorus bound in feed	Inorganic phosphorus	1 kg phytase product	30	EI'95	Full-scale	(17), peer reviewed journal paper
	Poultry feed production	Phytase			1 ton poultry feed	7.0	CML baseline 2000	Full-scale	(18), technical journal paper
	Poultry feed production	Protease	Hydrolyzes protein in the feed	Feed protein	1 ton poultry	11	CML baseline 2000	Full-scale	(19), peer reviewed journal paper[c]
Fine chemicals	Amino-butanoic acid production	Lipase	Aminolysis in (*S*)-3-aminobutanoic acid production	Chemicals, waste	1 kg (*S*)-3-amino-butanoic acid production	Environmental Factor reduced 8.8 times	E-Factor[e]	Lab-scale	(20), peer reviewed journal paper
	α-Naphthol production	Toluene ortho-monooxygenase	Oxidizes naphthalene to alpha-naphthol	Chemicals, waste	NA[d]	Yield improvement: 4.5 times	N/A[d]	Lab-scale	(21), conference paper
Pharmaceuticals	(*S*)-2-3-Dihydro-1H-indole-2-carboxlic acid production	Phenyl-alanine ammonia lyase	Formation of C—N bond	Chemicals, energy	1 kg product	155	IPCC	Lab-scale	(22), peer reviewed journal paper
	γ-Amino-butyric acid production	Lipolase	Resolution of cyanodiester	Chemicals, energy, waste	1 ton product	Environmental Factor reduced 5.1 times	E-Factor	Full-scale	(23), peer reviewed journal paper
	6-Aminopenicillanic acid production	Penicillin amidase	Deacylates penicillin molecule	Chemicals, energy	1 kg product	Environmental Index reduced 16 times	EI[f] (SuperPro designer)	Lab scale	(24), peer reviewed journal paper
	7-Aminopenicillanic acid production	D-Amino acid oxidase, glutaryl 7-ACA acylase	Oxidizes cephalosporin C salt and deacylates glutaryl 7-aminocephalosporic acid	Chemicals, energy	1 kg product	270	FLASC[g]	Full-scale	(25), peer reviewed journal paper
Cosmetics	Oleo chemical ester production	Lipase	Transesterification of vegetable oil	Chemicals, energy, raw material	5 ton myristyl myristate	940	EI'95	Full-scale	(26), technical journal paper[c]
Biodiesel	Methyl ester production	Lipase	Catalyzes the reaction of triglyceride and menthol to form methyl ester	Energy, chemicals, raw material	1 ton biodiesel	100	CML base 2000	Lab-scale	(27), peer reviewed journal paper
					1 ton biodiesel	2.5%	EI'99	Lab-scale	(28), peer reviewed journal paper

(1) Skals et al., 2008, (2) Kallioinen et al., 2003, (3) Fu et al., 2005, (4) Nielsen, 2006, (5) Nielsen and Høier, 2009, (6) Dettore, 2011, (7) Nielsen and Skagerlind, 2007, (8) Cowan et al., 2008, (9) Holm and Cowan, 2008, (10) DSM, 2009, (11) Oxenbøll and Ernst, 2008, (12) Oxenbøll et al., 2010, (13) Nielsen and Høier, 2009, (14) Kløverpris and Spillane, 2010, (15) Yon-Miaw, 2011, (16) Nielsen et al., 2008, (17) Nielsen and Wenzel, 2006, (18) Nagaraju and Nielsen, 2011, (19) Oxenbøll et al., 2011, (20) Weiß et al., 2010, (21) Osborne-Lee et al., 2008, (22) Poechlauer et al., 2010, (23) Dunn, 2011, (24) Biwer and Heinzle, 2004, (25) Henderson et al., 2008, (26) Thum and Oxenbøll, 2008, (27) Harding et al., 2007, (28) Jegannathan et al., 2011.

[a] Eco Indicator.

[b] Intergovernmental Panel on Climate Change.

[c] Background report followed ISO 14040/14044 standards with third-party peer review.

[d] Not available.

[e] Environmental Factor (ratio of waste over product).

[f] Environmental Index ('Environmental Factor' multiplied by 'Mass Index'. High 'Environmental Index' indicates high environmental impact).

[g] Fast Life Cycle Assessment of Synthetic Chemistry.

conventional bleaching process. Laccase use in the bleaching process saved elemental chlorine and implementation of the process reduced the contribution to ozone depletion and acidification, as well as reducing solid waste generation and energy consumption. However, flight transportation of a mediator used for the enzymatic process led to a net increase in contributions to greenhouse gas emissions and 'summer smog'.

3.3. Xylanase

Similar to laccase, xylanase enzyme can be used in the bleaching process. Xylanase degrades xylan in wood and facilitates lignin removal. The use of xylanase in chemical pulping reduces the need for bleaching chemicals (Bajpai, 1999; Skals et al., 2008). The LCA study by Skals et al. (2008) revealed that the reduced consumption of bleaching chemicals, especially ClO_2, saved a large amount of electricity, which in turn reduced the contribution to global warming and other energy-related impacts.

3.4. Lipase

In mechanical processing of pulp, the lipophilic materials present in wood form an insoluble deposit known as 'pitch' (Herbots et al., 2008) which lowers the quality of the paper and also hinders optimal operation of the paper machine. In the conventional process, cleaning agents and talc are added to logs to avoid pitch formation. In the enzymatic process, cleaning agents and talc are replaced by a lipase enzyme, which hydrolyzes lipophilic material, thereby saving chemicals and operation time (Hata et al., 1996). The LCA study by Skals et al. (2008) revealed that chemicals and energy saved by the lipase-assisted pitch control process reduced the net energy consumption, contribution to global warming and other energy-related impact categories.

3.5. Esterase

Agglomerates of glues (so-called 'stickies') are a major obstacle in recycled paper processing, because they cause holes and paper breaks, resulting in poor paper quality and frequent machine stops for cleaning. The conventional approach of 'stickies' control is by mechanical and chemical cleaning of operational equipment which leads to electricity, steam and solvent consumption. An alternative way of controlling stickies is to use an esterase enzyme, which hydrolyzes the polyvinyl acetate in the glues. Use of the enzyme reduces energy consumption during frequent production stops and reduces solvent use, thereby saving energy and chemicals (Patrick, 2004). An LCA study by Skals et al. (2008) shows that use of enzymes in 'stickies' control saves energy and reduces contributions to a broad range of environmental impacts.

4. Leather

Many accessories such as bags, shoes, furniture upholstery, etc. are made from leather produced from animal hides. Leather making involves several processes in which various harsh chemicals are consumed. Production and disposal of these chemicals after use is responsible for a large proportion of the environmental load of leather products (Marsal et al., 1999; Palanisamy et al., 2004). For this reason, various cleaner production methods including enzymes are being applied in the leather industry (Aravindhan et al., 2007; Li et al., 2010; Dettmer et al., in press). One enzyme application in the leather industry which has been subject to an LCA study is described in the following section.

4.1. Protease and lipase

Animal hide contains hair, unwanted protein and fat, which need to be removed before it is processed into leather. Conventionally, the unwanted protein and fat on the hide are removed by mechanical treatment in a drum with water, tensides and soda in a process called soaking, followed by a liming process, in which hair is digested by the action of sulfides. Protease and lipase enzymes are able to degrade protein and fat specifically, and can be used in the soaking and liming process as a supplement (Nielsen, 2006; Li et al., 2010). Using protease and lipase in soaking requires less processing time, tensides, and soda, resulting in electricity and chemical savings.

Nielsen (2006) used LCA to compare the environmental impact of conventional soaking and liming processes with enzyme-assisted processes. The study showed that environmental impacts of producing the enzyme were small compared with the impacts of the electricity and chemicals that were saved. The avoided production of sulfide, in particular, saved a considerable amount of energy. In addition the enzymatic process reduced wastewater pollution because hair was kept intact and not converted into organic matter in the wastewater as with the conventional process (UNIDO, 2000; Li et al., 2010).

5. Textiles

Raw cotton undergoes various processes such as scouring, bleaching, polishing and dyeing before it is converted into yarn and fabric. These processes consume large amounts of energy, water and chemicals, making the textile industry a resource-consuming and polluting industry (Santos et al., 2007). Cleaner processes are therefore given considerable attention in the textile industry in order to manage resource scarcity and pollution problems (NICE, 2012). A broad range of commercial enzymes are available for processing in the textile industry (Chen et al., 2007; Herbots et al., 2008) and enzymatic processes are seen as cleaner technologies which can supplement conventional processes (OECD, 2011). Some of the enzymatic solutions which have been subject to LCA studies are summarized below.

5.1. Pectate lyase

Raw cotton contains impurities such as wax and minerals bound to noncellulosic (pectin) components. These impurities hinder the dyeing operation in cotton fabric production and are removed through a process called scouring. Conventional scouring is a high-temperature chemical process which consumes large amounts of energy, water and chemicals such as hydrogen peroxide, sodium hydroxide and sodium carbonate. Pectate lyase is an enzyme which degrades pectin and is used in the scouring process to assist with the removal of impurities with lower energy and chemical consumption (Herbots et al., 2008). An LCA study by Nielsen et al. (2009) on the scouring process shows that the impact of enzyme production is low compared to the impact of energy, water, and chemicals that are saved.

5.2. Catalase

Knitted fabrics and yarns for light fabrics are bleached with hydrogen peroxide prior to dyeing and any hydrogen peroxide left on the fabric must be removed after the bleaching process to avoid interference with subsequent dyeing steps. Hydrogen peroxide is traditionally removed by rinsing the bleached fabric with hot water or by treating it with a reducing agent (sodium thiosulfate), consuming large amounts of energy and water and, in some cases, chemicals. A catalase enzyme is able to decompose hydrogen peroxide into water and oxygen at lower temperature in a single bath and thereby saving water, heat and electricity. An LCA study by Nielsen et al. (2009) on bleach clean-up shows that the impact of enzyme production is low compared to the impact of water, energy and chemicals that are saved.

5.3. Arylesterase

Similar to catalase, arylesterase enzyme can be used in the cotton bleaching process. The enzyme catalyzes the perhydrolyzes of propylene glycol diacetate and hydrogen peroxide to propylene glycol and peracetic acid. Peracetic acid acts as a bleaching agent under milder operational conditions than the traditional bleaching process, thereby saving energy and avoiding cotton loss. An LCA study by Dettore (2011) on the bleaching process shows that the environmental impact of an enzyme-assisted bleaching process is small compared to the environmental impact of a conventional bleaching process.

6. Detergent industry

Surfactants are a primary constituent of detergents which remove stains from clothes during laundry washing. Surfactants are most active at elevated temperatures and considerable amounts of energy are used to heat laundry water, particularly in cold countries. Moreover, surfactants released into the environment after washing are toxic to aquatic species unless they are removed in efficient wastewater treatment plants (Johnson and Marcus, 1996). Enzymes have the capacity to degrade stains at low washing temperatures and are less toxic than surfactants (OECD, 2011; Sekhon and Sangha, 2004) and are used as supplements in detergents. Nielsen and Skagerlind (2007) conducted an LCA study on a model detergent in which four enzymes (protease, lipase, amylase and cellulase) replaced three surfactants (ethoxylated alcohol, linear alkyl benzene sulfonate and sodium soap). The results show that the impact of enzyme production is low compared with the impact of surfactant production and that use of enzymes saves energy in the use phase and reduces contribution to aquatic toxicity in the disposal phase.

7. Food and beverage industry

Food and beverage products are major sources of environmental impact (Tukker et al., 2006; Olajire, in press) because they use large amounts of agricultural raw materials, energy and water. Growing populations and improving economies in many countries increase the demand for food and beverage products, and the food and beverage industry needs to reduce its environmental impact per unit of product produced (Spiertz and Ewert, 2009). Enzymes have been used in food and beverage production for many years for ingredient production (ETA, 2001) and yield improvement (Maria et al., 2007; DSM, 2009). Some of the enzymes that help to increase yield in the food industry and have been subject to LCA studies are summarized in the following sections.

7.1. Phospholipase

Crude vegetable oil contains phosphatide gums, which adversely affect the quality and stability of cooking oil and have to be removed by a process called degumming. The conventional degumming method runs at high temperature, uses caustic soda (Carr, 1976) and consumes large amounts of energy. Phospholipase is an enzyme which degrades phosphatide at low temperature. It simplifies the degumming process and increases the yield, thereby saving virgin vegetable oil, energy, water and caustic soda. An LCA study by Cowan et al. (2008) on the degumming process shows that the impact of enzyme production is low compared to the impact of materials and energy that are saved.

Another application of phospholipase is in mozzarella cheese production from cow's milk. A phospholipase hydrolyzes a phospholipid and generates a free fatty acid and a lysophospholipid. The lysophospholipid increases fat retention and thereby increases the yield of cheese. An LCA study by Nielsen and Høier (2009) shows that the use of phospholipase in cheese production saves milk and that the impact of enzyme production is small compared with the saved milk production (energy, etc. for feed production plus CH_4 and N_2O emissions from dairy farms).

7.2. Lipase

Hard stock is a main component in margarine, which is produced by interesterification of vegetable oil. The conventional interesterification process runs at elevated temperatures and is catalyzed by an inorganic catalyst of sodium methoxide. The process uses a large amount of energy for heating and generates an undesired dark by-product which needs to be removed in a series of bleaching steps. Alternatively, an immobilized lipase enzyme can be used as catalyst in the interesterification of oils. The process is highly specific, runs at low temperature and generates less by-product (Holm and Cowan, 2008). A comparative LCA study by Holm and Cowan (2008) on two interesterification processes shows that impacts caused by the enzymatic process are small compared with the conventional process because energy was saved and yield of hard stock was improved.

7.3. Pectinase

Pectin is a complex polysaccharide which is found in fruits. It has a negative impact on yield when fruits are converted to juice and is responsible for haze and precipitate formation in juice (Whitaker, 1984). Pectinase is an enzyme which hydrolyzes pectin and is used to clarify juice and to increase yield in juice production. An LCA study on apple juice production shows that pectinase use saves fruit and that CO_2 savings in the application is 18 times higher than the emission (DSM, 2009).

7.4. Amylase and lipase

Starch is a main constituent of bread and bread becomes hard and unpleasant to eat with age because the starch crystallizes. The addition of amylase and lipase enzymes in bread-making reduces the crystallization of starch in the bread and extends the shelf life, thus reducing bread wastage. LCA studies by Oxenbøll and Ernst (2008) and Oxenbøll et al. (2010) on white bread and Chinese steamed bread respectively show that the environmental impact caused by enzyme production is small compared to the environmental impact avoided by saving bread. The savings were primarily driven by avoided grain production and bread transportation.

7.5. Amylase and protease

A key process in beer production is fermentation, in which sugars are converted into alcohol. Sugars are traditionally produced from starch from various grains by allowing it to react with enzymes from germinated barley (malt) in a process called mashing (Takamoto et al., 2004; Saxe, 2010). Alternatively, starch can be converted into fermentable sugars with a mixture of industrially produced enzymes (amylase, protease etc.) and the malting process can be bypassed. Production of malt consumes grain as well as heat for drying, and avoiding the malting process saves energy and agricultural land. LCA studies by Kløverpris and Spillane (2010) and Yon-Miaw (2011) show that environmental impacts caused by enzyme production are small compared with the impact avoided by saving energy and barley for malt production.

8. Animal feed

Some constituents of animal feed are not degradable by livestock and therefore energy, protein and minerals are not fully utilized. Feed and mineral consumption may therefore be higher than necessary in order to produce a certain amount of meat. Production of feed and inorganic supplements is energy-intensive and the undigested nutrients excreted by the livestock into the environment lead to pollution problems (FAO, 2004; FAO, 2007; Elferink et al., 2008; Tongpool et al., 2012). Enzymes are capable of degrading complex components in the feed, and the addition of industrially produced enzymes increases the energy and nutrient value of feed and reduces emissions into the environment (Aehle et al., 2008; Monsan and Donohue, 2010). Enzymes used in the animal feed industry which have been subject to LCA studies are summarized in the following sections.

8.1. Xylanase

Xylan is a dietary fiber present in cereal cell walls and is an energy rich constituent of animal feed. However, its complex polymer structure makes it indigestible to monogastric animals (pig and poultry, etc.) and energy and protein in the feed is not fully utilized. Production of feed consumes agricultural land, water and energy and has an impact on the environment. Xylanase is an enzyme which depolymerizes xylan into digestible smaller units and increases the energy and protein value of the feed (Monsan and Donohue, 2010), thereby reducing the feed requirement per unit of animal product produced. An LCA study by Nielsen et al. (2008) on pig production shows that the environmental impact of the enzyme is small compared with the avoided impact achieved by saving feed and reducing emissions of N_2O and CH_4, etc. from manure.

8.2. Phytase

Phosphorus is an important macronutrient for animal growth and inorganic phosphorus is often added to animal feed in intensive livestock production. Phosphorus occurs naturally in many feed ingredients but only parts of it are available to monogastric animals because phosphorus is bound in phytate. Inorganic phosphorus supplements are produced from precious phosphate rock in an energy consuming process and the release of undigested phosphorus into the environment via livestock manure leads to nutrient enrichment of water bodies. Phytase is an enzyme which hydrolyzes phytate and can be used to release existing phosphorus in feed (Selle et al., 2000; FAO, 2010; Monsan and Donohue, 2010), thereby reducing the need for inorganic phosphorus supplementation. An LCA study by Nielsen and Wenzel (2006) and Nagaraju and Nielsen (2011) on pig and poultry feed production respectively shows that the environmental impact caused by the enzyme is small compared with the impact of inorganic phosphorus because a small amount of enzyme replaces a large amount of monocalcium phosphate (MCP) and a considerable amount of phosphorus is avoided in the manure.

8.3. Protease

Protein is an important building block in livestock production and animal feed for intensive animal production often contains large amounts of protein rich ingredients. Only parts of the protein contained in feed are digestible, however, and the undigested protein is responsible for releasing nitrogen compounds into the environment via manure. Proteases are enzymes which hydrolyzes protein and can be used to increase the digestibility of protein, for instance in poultry feed, thereby reducing nutrient release to the environment (Marquardt et al., 1996; Nahm, 2007; Herbots et al., 2008). An LCA study by Oxenbøll et al. (2011) on poultry feed production shows that the environmental impact of the enzyme is small compared with the avoided impact achieved by reducing N_2O and ammonia emissions, etc. through the poultry litter.

9. Fine chemicals

The fine chemicals industry plays a key role in supplying pure enantiomer compounds to the pharmaceutical and cosmetic industries, etc. Enantiomers are traditionally produced through synthetic routes by resolutions of racemic mixtures using chemical catalysts (Schulze and Wubbolts, 1999; Sheldon, 2011). However, synthetic routes involve many intermediate reactions and consume large amounts of raw materials, energy and solvents, as well as generating problematic waste. Highly specific enzymes can be used as alternatives to the chemical catalysts and use of enzymes saves energy, chemicals and waste generation due to the high specificity and milder reaction conditions (Straathof et al., 2002; Kuhn et al., 2010; BASF, 2008). The following section provides an insight into enzymes used in fine chemicals production which have been subject to EIA studies.

9.1. Lipase

β-Amino acids are important building blocks for the synthesis of pharmaceuticals. In the category of β-amino acids, (*S*)-3-aminobutanoic acid is traditionally produced from (*E*) ethyl but-2-enoate using a chemical catalyst in a multi-step reaction consuming large amounts of solvent and toxic chemicals. An immobilized lipase can supplement the existing chemical catalyst in (*S*)-3-aminobutanoic acid production, however (Weiß et al., 2010), reducing the number of production steps and avoiding column chromatography separation of products and by-products. This saves solvents and reduces the amount of toxic waste. An EIA study by Weiß et al. (2010) on (*S*)-3-aminobutanoic acid production shows that the Environmental Factor (E-factor) of the enzymatic process is small compared to the E-factor of the conventional process.

9.2. Toluene ortho-monooxygenase

α-Naphthol is a key intermediate in the manufacture of dyes, herbicides, insecticides and pharmaceuticals. α-Naphthol is traditionally produced by hydrogenation and oxidation of naphthalene using a $CoMo{-}Al_2O_3$ catalyst at high temperature and pressure. Toluene ortho-monooxygenase is an enzyme which can be used to oxidize naphthalene at low temperature and pressure, thereby saving energy and increasing the product yield. An EIA study by Osborne-Lee et al. (2008) on α-naphthol production shows that emissions of chemicals and the ‘risk index’ of the enzymatic process are negligible compared to emissions of chemicals and the ‘risk index’ of the conventional process.

10. Pharmaceuticals

Synthesis of pharmaceutical ingredients involves several reaction steps and unit operations, which consume energy and chemicals, generate hazardous waste and lead to environmental impact

(Ran et al., 2008). Pharmaceutical companies are therefore under pressure to develop and implement environmentally friendly processes (Woodley, 2008; Valavanidis and Vlachogianni, 2012). The application of enzymes in pharmaceutical ingredient synthesis has the potential to reduce environmental load (Pollard and Woodley, 2007) and enzymes which have been subject to EIA or LCA studies are summarized in the following sections.

10.1. Phenylalanine ammonia lyase

(*S*)-2-3-Dihydro-1H-indole-2-carboxylic acid is a key intermediate for antihypertensive drugs (De Vries et al., 2011). The traditional route of (*S*)-2-3-dihydro-1H-indole-2-carboxylic acid production is a seven-step process which consumes large amounts of raw materials and energy and generates waste. Phenylalanine ammonia lyase is an enzyme which can be used along with a copper catalyst to produce the product in a three-step process, thereby reducing chemical and energy consumption, etc. An LCA study by Poechlauer et al. (2010) on (*S*)-2-3-dihydro-1H-indole-2-carboxylic acid production shows that the CO_2 footprint of the enzymatic process is small compared to the CO_2 footprint of the traditional process.

10.2. Lipase

γ-Aminobutyric acid (GABA) is a drug which is used for disorders of the nervous system. GABA is traditionally produced from β-cyanodiester and (*S*)-mandelic acid using a nickel catalyst and the process consumes large amounts of raw materials, catalyst, chemicals and solvents such as methanol, ethanol, etc. A lipase can be used along with the nickel catalyst, however, to increase the reaction speed and product yield. An EIA study by Dunn (2011) on GABA production shows that the E-factor of the chemo-enzymatic process is small compared with the traditional process.

10.3. Penicillin amidase

6-Aminopenicillanic acid (6-APA) is an intermediate product for antibiotic production. 6-APA is produced from penicillin G in a three-step process and consumes energy and chemicals such as phosphorus pentachloride, dichloromethane, ammonia, etc. Penicillin amidase is an enzyme which can be used as catalyst in 6-aminopenicillanic acid production in a single-step process, thereby saving energy and chemicals. An EIA study by (Biwer and Heinzle, 2004) on 6-APA production shows that the 'Environmental Index' and 'Mass Index' of the enzyme-catalyzed process are small compared to the conventional process.

10.4. D-Amino acid oxidase and glutaryl 7-ACA acylase

Similar to 6-APA, 7-aminocephalosporic acid (7-ACA) is an intermediate product for antibiotic production. In the traditional route, the 7-ACA is synthesized from a potassium salt of cephalosporin C in a four-step process consuming energy and chemicals such as methanol, PCl_3, dimethylanaline, etc. D-amino acid oxidase and glutaryl 7-ACA acylase are enzymes which can be used as catalysts in 7-ACA production in a three-step process, thereby saving energy and chemicals. An LCA study by Henderson et al. (2008) on 7-ACA production shows that the environmental impact caused by the enzymatic route is small compared with the impact of the chemical route.

11. Cosmetics

Some of the ingredients used in the cosmetic industry are produced by chemical catalysis of plant or petrochemical-based raw materials. The processes are run at high temperatures and considerable amounts of by-products are generated due to the unspecific action of the catalysts. Specific enzymes can replace the chemical catalyst, however, improving yields and reducing by-product and waste generation (Hills, 2003; Mohorčič et al., 2007; Ghoul and Chebil, 2012). One enzymatic solution in the cosmetic industry which has been subject to an LCA study is summarized in the following section.

11.1. Lipase

Emollient esters are oleochemicals which are widely used in cosmetic products due to their emollient and moisturizing properties, etc. (Keng et al., 2009). Emollient esters are traditionally produced by transesterification of vegetable oil and alcohol using tin oxalate as catalyst at elevated temperature. This process consumes tin catalyst, energy and chemicals such as sulfuric acid and calcium hydroxide and the process generates considerable amounts of waste. A lipase can replace the tin catalyst in the transesterification process, however, saving energy, raw materials, auxiliary agents and waste because the lipase acts specifically at low temperature. An LCA study by Thum and Oxenbøll (2008) on an emollient ester (myristyl myristate) shows that the environmental impact caused by the enzymatic process is small compared with the conventional process.

12. Biodiesel

Fatty acid alkyl ester produced from vegetable oil and alcohol, known as biodiesel, can be used as an alternative to fossil-based diesel. Fossil fuel depletion sometime in the future and growing energy prices have made biodiesel an interesting alternative fuel that is being introduced to the transport sector in many countries (Tzimas et al., 2004; Demirbas, 2007). An enzymatic solution in biodiesel production which has been subject to an LCA study is summarized in the following section.

12.1. Lipase

Conventional biodiesel is produced by transesterification of triglycerides from vegetable oil and an alcohol (methanol or ethanol) using an alkali catalyst. The process is carried out at elevated temperature and consumes heat and chemicals such as NaOH (catalyst) and sulfuric acid (neutralizing agent), while side reactions lead to soap formation. An immobilized lipase can degrade triglycerides and can be used as an alternative catalyst in the transesterification process. The enzymatic process is carried out at low temperature, saving energy and chemicals and avoiding soap formation (Fukuda et al., 2001; Noureddini et al., 2005). LCA studies by Harding et al. (2007) and Jegannathan et al. (2011) on biodiesel production show that the environmental impact of the enzymatic process is small compared to the conventional process.

13. Summary and discussion

The 28 comparative environmental assessment studies of enzymatic processes versus conventional processes which have been reviewed in this paper are summarized in Table 1. The table shows the broad variability of enzyme applications in industrial production, and demonstrates that savings on raw material energy and/or chemicals obtained by implementation of enzymatic processing are reducing greenhouse gas emissions in all case studies except a preliminary study on bleaching in pulp and paper industry.

In the following section, a range of characteristics of the studies will be discussed and recommendations for future development of environmental assessments of enzymatic solutions will be given.

13.1. Impact category selection

Impact category global warming is addressed in 80% of the papers reviewed, followed by energy use and acidification (65%), eutrophication and photochemical ozone formation (50%). Agricultural land use is only addressed in 30% of the studies and toxicity and water consumption are addressed in less than 10% of the studies.

Water consumption for industrial production is growing in line with the improving economies in many countries, and clean water is becoming a scarce resource in many places (UNEP, 2012). We recommend that the water saving potential and wastewater toxicity reduction potential of enzymatic solutions be given more attention in future environmental assessments.

Agricultural land use may be influenced both positively and negatively by the implementation of enzymatic processes in production. Positive effects have been observed in cases where agricultural raw materials are saved (Nielsen et al., 2008; Thum and Oxenbøll, 2008; Kløverpris and Spillane, 2010), whereas agricultural land use has become as a trade-off in cases where only fossil fuels and/or inorganic chemicals are saved. The latter is because agricultural raw materials such as sugar are used to feed microorganisms in enzyme production and no agricultural land use is avoided when the enzymes are used (Skals et al., 2008; Nielsen et al., 2009). Skals et al. (2008) studied the trade-off between agricultural land use for enzyme production and GHG savings achieved in the pulp and paper industry and showed that GHG savings ranged from 290 to 11,000 tons of CO_2 equivalents per ha year. This is at least a factor of 10 higher than CO_2 savings achieved by converting biomass directly to energy, thus justifying the agricultural land use. We recommend that agricultural land use be addressed to greater extent in future environmental assessments.

Seven percent of the studies cited in this paper are so-called 'carbon footprint' studies, which focus solely on effects on the climate. We find that carbon footprint assessments are excellent for preliminary assessments and encourage researchers to include more impact indicators in full assessments in future studies to ensure that no important benefits or trade-offs of enzyme technology are overlooked.

13.2. Uncertainty and variation

Uncertainty and variation has received considerable attention in some studies and little or no attention in others. Studies which have dealt with uncertainty and variation in detail have used 'qualitative data quality assessments', 'sensitive analysis' or 'What if' analysis. It is generally concluded that the variation and uncertainty of results are considerable but that the overall observation that enzymatic processes are environmentally sound compared with the conventional processes that they replace is robust. The same appears to apply for studies where uncertainty and variation is ignored or given less attention because the difference in environmental impact of conventional and enzymatic processes is considerable. We encourage researchers to address uncertainty and variation in more detail in future environmental impact studies of enzyme technology to avoid any doubt about the robustness of observations.

13.3. Critical review

Fifty percent of the LCA studies cited in this paper were conducted in accordance with ISO 14040 standards (ISO, 2006) and critical review has been used extensively to justify the appropriateness of methodology, technical validity, interpretations and transparency. EIA studies have generally not been subjected to critical review according to the applied standards but most have been subject to journal peer review. Critical review is important to avoid bias and ensure confidence in the final results and we encourage that the current high standard on peer review is maintained and that information about applied standards and type of peer review is communicated clearly with the results.

13.4. LCA versus EIA

The majority of studies (86%) reviewed in this paper used LCA as an environmental assessment tool, while the remaining 14% studies (primarily from the fine chemicals and pharmaceuticals industries) used EIA. EIA is advantageous compared to LCA because of its simplicity. However, EIA has two important drawbacks compared to LCA: 1) it focuses on a single process and ignores upstream production of items such as raw materials and chemicals; and 2) it uses single metrics such as 'Environmental Factor' or 'Environmental Index', and does not differentiate between different types of effects on the environment. We find that using LCA gives a more complete picture of the environmental aspects of enzyme application than EIA and encourage actors in this field to move toward LCA instead of EIA for future environmental impact studies of enzyme technology.

13.5. Geographical distribution of LCA studies

Environmental impacts of implementing enzymes in a process vary from region to region because production technology, energy supply, transport systems, agricultural practices and ambient temperatures vary. Most of the environmental assessment studies reviewed in this paper (74%) are specific to Europe, while a few are specific to areas such as the United States (5%) and China (15%). Much industrial production takes place outside these countries and we encourage researchers to develop LCA databases specific to other regions and to extend the use of LCA to more countries.

14. Conclusion

Enzymatic processes have a wide range of applications across several industries such as household care, food, animal feed, technical industries, fine chemicals and pharma. The unique properties of enzymes such as high specificity, fast action and biodegradability allow enzyme-assisted processes in industry to run at milder reaction conditions, with improved yields and reduced waste generation. This review summarizes 28 comparative environmental assessments conducted over the past 15 years, and the results show that implementing enzymatic processes in place of conventional processes generally leads to reduced contributions to global warming and also reduced contributions to acidification, eutrophication, photochemical ozone formation and energy consumption to the extent that this has been investigated. Only one-third of the studies have addressed 'agricultural land use' as an impact category. However, agricultural land use savings appear to occur in industries where enzymatic processes save agricultural raw materials, whereas this becomes a trade-off in processes where only fossil fuels and/or inorganic chemicals are saved. Agricultural land use appears to be justified by other considerable environmental improvements, and the results of this review support the hypothesis that enzyme technology is a promising means of moving toward cleaner industrial production.

15. Outlook and recommendations

The present study shows that the use of enzymatic processes in industry is a promising means of moving toward cleaner industrial production. However, barriers such as lack of knowledge of enzymatic processes, traditional thinking among manufacturers and suppliers, and governmental bureaucracy during approval of new solutions in many countries tend to delay broader implementation.

A number of steps should to be taken to overcome these barriers and accelerate the harvesting of environmental benefits offered by enzyme technology:

1) Increase education on biotechnology and enzymatic processes.
2) Create awareness of biotechnology through workshops, seminars and best practice schemes.
3) Increase sustainability stakeholder collaboration in product chains (raw materials producers, product manufacturers, retailers, consumers and investors).
4) Increase sustainability target-setting on corporate social responsibility and report progress in sustainability indexes.
5) Continue documenting environmental impacts of new and existing biological solutions.
6) Streamline public approval of new biotechnological solutions.
7) Increase openness on production and use of enzymes in industry.

In addition, governments could consider phasing out subsidies to fossil fuels and implementing green tax schemes (United Nations, 2012) to increase competitiveness of the most energy and resource efficient technologies in future.

Conflict of interest

The authors are employed by Novozymes A/S and have an interest in promoting enzymatic processes because the company produces and markets enzymes and other biological products.

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