Industrial Enzymes for Sustainable Bio-Economy: Large Scale Production and Application in Industry, Environment, and Agriculture in Eastern Africa

Amare Gessesse, Francis Mulaa, Sylvester Leonard Lyantagaye, Laetitia Nyina-Wamwiza, Bo Mattiasson, Ashok Pandey

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International Livestock Research Institute

P O Box 30709, Nairobi 00100, Kenya
Phone + 254 20 422 3000
Email ILRI-Kenya@cgiar.org

P O Box 5689, Addis Ababa, Ethiopia
Phone + 251 11 617 2000
Email ILRI-Ethiopia@cgiar.org

www.ilri.org

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Principal Investigator:

Dr.Amare Gessesse
Biotechnology Program, College of Natural Sciences, Addis Ababa University
P. O. Box 1176, Addis Ababa, Ethiopia
Tel. +251-911-146855 Cell. +251-911-146855
E-mail: amare.gessesse@gmail.com

Co-Investigators:

Prof. Francis Mulaa
Department of Biochemistry, University of Nairobi
P.O. Box 30197, Nairobi, Kenya
Tel:+ 254-020-4442841 Fax:+254-020-4441186
E-mail: mulaafj@uonbi.ac.ke mulaafj@yahoo.com

Dr. Sylvester Leonard Lyantagaye
Department of Molecular Biology and Biotechnology, College of Natural and Applied Sciences, University of Dar es Salaam,
P.O. Box 35179, Dar es Salaam, Tanzania.
Tel: +255) 787537030 / (+255) 712569527, E-mail: lyantagaye@amu.udsm.ac.tz , slyantagaye@gmail.com

Dr. Laetitia Nyina-Wamwiza
Department of Animal Production, Faculty of Agriculture and
Department of Biology, Faculty of Science
National University of Rwanda
P. O. Box 117, Butare, Rwanda
Mobile +250 0788526183
E-mail: nyinawamwiza@yahoo.fr, lnyinawamwiza@nur.rw

Prof. Bo Mattiasson
Department of Biotechnology, Lund University
P.O. Box 124, S-221 00, Lund Sweden
Email:Bo.Mattiasson@biotek.le.se

Prof. Ashok Pandey
Center for Biofuels & Biotechnology Division
National Institute for Interdisciplinary Science and Technology
Trivandrum-695 019, India
Tel:+91-471-251 5279 Fax: +91471 249 5959
Email:pandey@niist.res.in: ashokpandey56@yahoo.com

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SUMMARY

To date Africa's participation in the global economy is largely confined to supplying raw materials. Adding value to these raw materials is expected to lead to economic growth and improvement in the standard of living. In Eastern Africa, because of availability of raw materials, developing capacity in the leather, textile, pulp and paper, detergent, and starch industries is believed to offer competitive advantages. However, some of these industries are highly associated with environmental pollution.

Recently developments in industrial biotechnology, defined as the use of enzymes or microorganisms for industrial processes, has offered a viable option to decrease or avoid environmental pollution from such industrial activities. Widespread use of enzymes in industrial processes, in addition to lowering the level of pollution, could lead to improvement in product quality and/or process efficiency. Thus, availability of enzymes locally with affordable price and with expert support on their use is expected to have significant contribution in the region by lowering environmental pollution and by replacing several imported chemicals as processing aids. Furthermore, because of the availability of extremely unique habitats, such as alkaline environments, hot springs, etc with huge microbial diversity, the region could be, in the long term, highly competitive in the global industrial enzyme market. For example, one enzyme isolated by Genecor, an American biotech company, from a Kenyan soda lake was estimated to earn the company over US $600 million annually.

In the last few decades, through research conducted in the different institutions several novel microbial strains producing potentially attractive enzymes for industrial application were isolated and characterized. Cultivation conditions for these organisms have also been optimized. Evaluation of some of these enzymes under application conditions gave extremely encouraging results. Given the importance of these enzymes in serving as processing aids in different industries in the region and their role in significantly reducing environmental pollution, scaling up of production processes and use of the enzymes at industrial scale is felt absolutely essential. The main objectives of this study are therefore, to scale up production, optimize enzyme stabilization and formulation, and test the enzymes under application conditions.

Enzyme producing microbial strains earlier isolated will be grown in large scale using solid state fermentation or submerged fermentation. The enzymes will be concentrated, stabilized, and formulated for industrial application. These enzymes will then be used for leather processing, textile processing, protein hydrolysis, detergent formulation, as animal feed additive, pulp bio bleaching, etc. Testing will be carried out at factory settings in different factories in the region. For products where enzymes are already in use (such as bating agents in the leather industry) the new enzymes will be compared with commercial enzymes and the best enzyme selected and promoted for use in the region. For processes where enzymes are not used (usually for reason of cost) factories will be encouraged to adopt the technology by giving them free samples.

The technology developed will then be popularized through different channels. A workshop will be organized for enzyme users in the region and different industries will be encouraged to use these products. Similarly workshops will be organized for business people in the region to attract their attention and encourage them to invest in this technology. A company specialized in the production of industrial enzymes in partnership with private sector (and if necessary foreign partners) will be established.

Successful implementation of this project is expected to help the region to develop the industrial sector with little or no environmental pollution. As Africa's microbial biodiversity is unique, in the medium to long term, the region could gain access to a significant slice of the global enzyme market.

1. BACKGROUND

The 20th century has witnessed remarkable growth and expansion in industrial output which provided jobs and income, goods and services, and opportunities to improve the standard of living for millions of people in many countries. However, Africa was not benefiting from this industrial development and its participation in the global economy is largely confined to supplying raw materials. Adding value to these raw materials is believed to have enormous economic contribution and help to alleviate poverty in the continent. Therefore, at present there is a growing desire to expand industrial development to bring about sustained economic growth. However, lesson learnt from developed countries showed that waste generated from industrial activities result in pollution of air, water, soil, and emit massive amounts of greenhouse gases which are responsible for climate change. To reduce further deterioration of the global environment it is important to reduce the amount of waste and pollution generated through industrial activities. But if developing countries follow the same path of industrialization as developed countries this goal cannot be achieved easily.

In this respect, recent developments in industrial biotechnology has offered an alternative approach for the reduction (or in some cases total elimination) of pollution from many industrial sectors without affecting production efficiency and product quality. Industrial biotechnology is defined as the use of enzymes or microorganisms for the production of goods and services. At present enzymes find increasing application in many industrial processes. As a result the global industrial market is growing very fast with a current estimated value of US$7 billion. Although enzymes are found in all living organisms, most industrial enzymes currently in use are obtained from microorganisms. Worldwide over 120 companies are known to produce industrial enzymes and more than 80% of the companies controlling up to 90% of the market are located in Europe and North America with none in Africa. But Africa has a huge potential for the discovery of novel enzymes that could prove highly useful in different industrial processes.

The East African region is endowed with unique microbial diversity which could serve as a source of novel enzymes for industrial application. Already some enzymes with an attractive potential for industrial application have been discovered from the region (Pennisi 1997; Gessesse, 1998; Mamo and Gessesse 1999; Gessesse *et al*. 2003; Yihun, 2007; Kebede 2008; Hashim *et al* 2005; Damte, 2011; Seid, 2011). Despite its huge potential for biotechnology innovation, to date the region make no use of this resource. In some cases, for example in Kenya, multinational companies from developed countries have benefited from the resource. A case in point is the American enzyme producer, Genecor which developed an alkaline cellulase enzyme from an alkaliphilic *Bacillus* sp isolated from a soda lake in Kenya. This enzyme was used by Procter & Gamble in its detergent called Tide (Sheridan, 2004). Experts estimate that the annual sale of the enzyme by Genecor to be in excess of US$600 million (Pennisi 1997). This case initiated a series of legal battle between the Kenya Wildlife Service (KWS) and the two companies using this enzyme.

But considering the huge microbial biodiversity that exist in the region, several enzymes of tremendous potential for industrial application are still awaiting discovery. Therefore, to use this resource for the benefit of the region, it is important that enzyme producing companies emerge in the region and start to compete in the global industrial enzyme market. Production of industrial enzymes at commercial scale in the region will have several advantages. First local availability of such enzymes in sufficient quantity and with affordable price could encourage local industries to adopt industrial biotechnology and release less waste which could help to reduce or avoid environmental pollution. Secondly, it could help Africa to share a significant proportion of the growing global industrial enzyme market and generate job. Thirdly, it could play an important role in laying the foundation for the development of industrial biotechnology in the region. This is important when one considers the fact that Africa is still in the process of growing its industrial manufacturing sector.

At present industrial enzymes are becoming extremely important for use in industry, agriculture, and environmental protection (Fig. 1). The most important industries which currently use industrial enzymes include leather tanning, textile, pulp and paper, chemical and pharmaceutical, food, detergent, and starch industries.

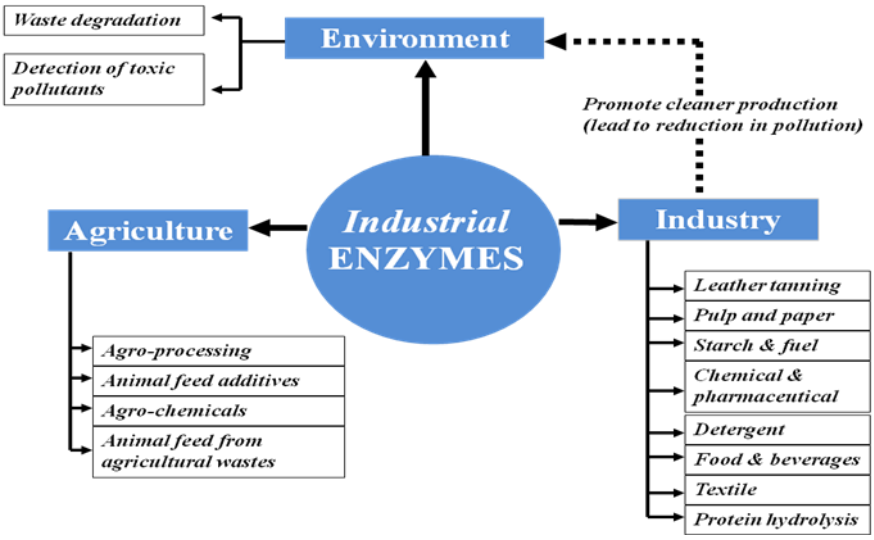

Fig. 1: Examples of application of enzymes in industry, agriculture, and the environment

2. IMPORTANT INDUSTRIES IN EASTERN AFRICA: environmental performance and economic contribution

Because of availability of raw materials the East African region has ample potential for growth and expansion of such industries as leather tanning, textile, pulp and paper, starch, detergent, biofuel, chemical etc. At present, compared to the other industries, there has been relatively better effort, at least in some countries in the region, to expand the leather tanning and textile industries. But, there is still a huge potential for the expansion of these and other industries.

For example Eastern Africa is well known for its large number of cattle with Ethiopia, Sudan, and Tanzania, respectively, ranking as first, second and third in the continent. Export of raw and semi-processed skin and hides has been a major foreign currency earning activity in the region. In recent years, governments in the region encourage leather industries to finish processing of skins and hides to finished leather and leather goods.

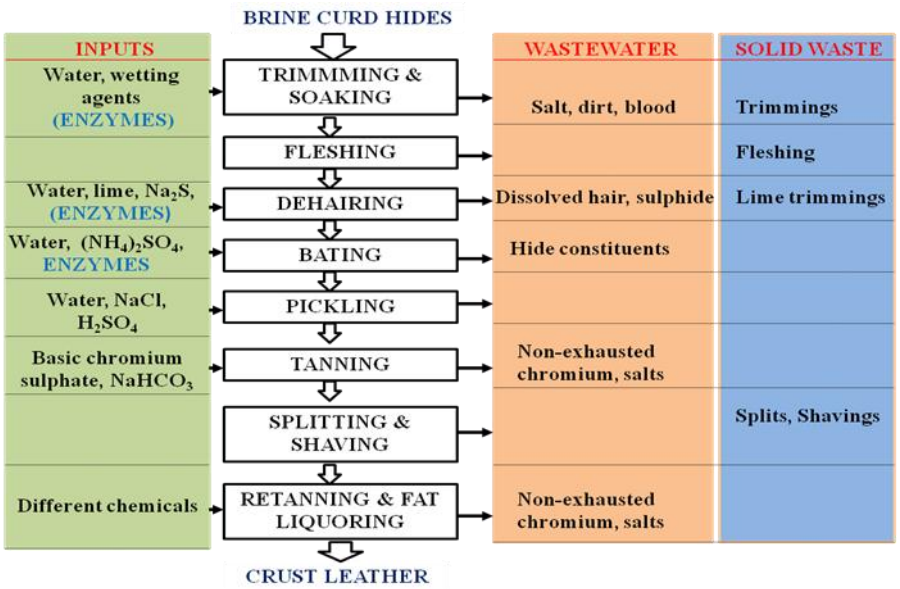

Fig. 2: Flow chart of leather tanning process

In Ethiopia there are about 25 leather tanning industries, many of them engaged in the production of finished leather. In Tanzania more than a dozen tanneries are currently in operation. In these factories hundreds of thousands of people work and export of leather and leather goods is an important source of foreign currency. However, the leather tanning industry is also well known for its negative impact on the environment. Leather tanning process involves using different chemicals, such as sulfides, chromium, lime, salts, etc., and releases huge quantities of solid and liquid waste (Fig. 2). As a result leather tanning industries are negatively associated with severe environmental pollution.

Similarly, because of its potential to grow large quantities of cotton, the region has an enormous potential for the growth and expansion of the textile industry. Like the leather industry the textile industry also use different chemicals and release large quantities of toxic waste. Here too industrial enzymes, such as amylases, cellulases, peroxidases, and catalases proved very important in bringing about process efficiency and reduction in the amount of environmental pollution (Fig. 3).

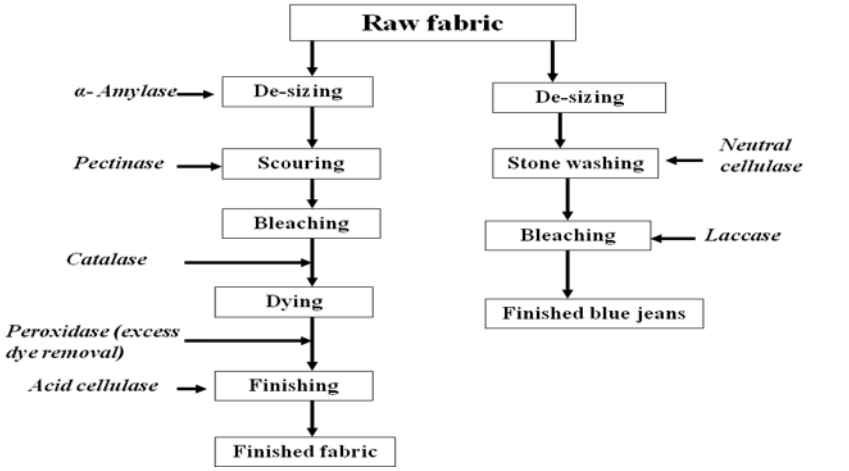

Fig. 3: A schematic outline of textile processing and application of enzymes in the textile industry

In recent years Africa was given tariff free export of selected products in the USA and EU markets. This was expected to boost economic growth and expansion of the textile and leather industries in the region and bring about sustained economic growth. This didn't yet happen as expected for a number of reasons. One obvious reason is failure to meet stringent standards of developed countries. Another potential area of concern, at least in the future, is that in recent years many importers in developed countries make environmental performance of a factory as a mandatory condition before importing products from developing countries. Thus in order to access developed county markets, industries in the region need to care for the environment. Therefore, availability of industrial enzymes as processing aids in the region is expected to help local industries lower environmental pollution and enable them to be competitive in the global market.

3. ENZYMES OF POTENTIAL INDUSTRIAL IMPORTANCE FROM PREVIOUS STUDIES

In the last two decades Addis Ababa University, Ethiopia and University of Nairobi, Kenya received substantial funding from Sida/SAREC and other donors (such as NUFU of Norway; DFID, UK, etc) for research and training in the area of industrial biotechnology. Through these projects at Addis Ababa University 5 PhD and more than 20 MSc students did their thesis research in the area of enzyme technology. Similarly in Kenya 4 PhD students did their thesis research on enzyme technology.

In addition to building capacity in industrial biotechnology (both in terms of human capacity and infrastructure) over the years through these studies several unique microbial strains producing enzymes of enormous potential for a variety of applications were isolated and characterized. Some of the enzymes were unique that patent applications have already been filed (Hatti-Kaul *et al*, 2006). The potential application of some of these enzymes in the leather tanning, starch processing, detergent, and protein hydrolysis industries were tested and very encouraging result was obtained. Furthermore, growth and enzyme production using cheap substrates either using SmF or SFF was developed and optimized.

We strongly believe that scaling up enzyme production and stabilization for industrial scale use of these enzymes could bring about tremendous environmental and economic benefit to the region. The following are the different enzymes that we studied in detail and the different industries that could benefit from their use.

3.1. Bacterial alkaline proteases

Proteases are extremely useful enzymes with enormous application in the detergent, leather tanning, protein hydrolysis, chemical, and other industries. Currently alkaline proteases used for detergent application are known to account for 25% of the global industrial enzyme market. As shown in Fig. 2, proteases are also extremely useful in the leather industry where they are important for soaking, dehairing, and bating application. Today use of enzymes for leather bating is mandatory and all leather industries in the region import proteases as bating agents with the expenditure of foreign currency.

Over the years several microbial strains producing novel proteases were isolated from the East African region. To date at Addis Ababa University a total of 8 graduate students did their thesis research on alkaline proteases and more than a dozen strains producing novel proteases were isolated, characterized, and their growth and production optimized. Two of the strains designated as *Bacillus pseudofirmus* AL-89 and *Nesterenkonia aethiopica* AL-20 were grown using chicken feather as sole source of nitrogen and carbon (Gessesse *et al.* 2003). Feather is a byproduct of the poultry industry and its accumulation around processing sites often cause serious environmental concerns. Though it consists of more than 90% protein, because of its resistance to enzymatic digestion feather cannot be used as animal feed and its disposal is often problematic. Therefore its use for enzyme production is extremely attractive. Many experts estimate that up to 40% of the production cost of industrial enzymes is accounted for by the growth substrate. Hence, use of feather for protease production could on the one hand allow production of value added chemicals (enzymes) and on the other hand it could reduce the environmental impacts of feather waste.

The protease produced by *N. aethiopica* AL-20 is very unique in many ways and it has very interesting application as detergent additive. To test its potential use as detergent additive the enzyme was incubated in the presence of commercial detergents and its stability was compared with different known enzymes, including the endogenous enzyme added by the detergent manufacturer. As shown in Fig. 4, protease AL-20 was very stable in the presence of commercial detergent retaining 100% of its original activity after 1 h incubation at 60ºC. All the other enzymes rapidly lost their activity. For example, in one of the detergents tested (Areal) the endogenous enzyme lost over 60% of its original activity in 10 min and 100% of its original activity in 40 min (Fig. 4). Another extremely important property of this enzyme is that it does not require calcium for stability. All other microbial proteases require calcium for stability. On the other had detergent formulations contain chelating agents to decrease water hardness. Thus at temperatures above 50 ºC enzyme stability is greatly reduced. From commercial point of view, detergent proteases that do not require calcium are so important that several researchers put a lot of effort in achieving this property using protein engineering techniques. This is because, if the enzyme does not require calcium for stability, the detergent manufacturer can add less enzyme and this will greatly lower the production cost of the detergent. To date protein engineering techniques failed to achieve a functional calcium independent enzyme. But in our laboratory we have an enzyme ‘engineered’ by nature to be calcium independent and highly active in commercial detergents. Its production at commercial scale is therefore expected to give a competitive advantage in the global detergent enzyme market.

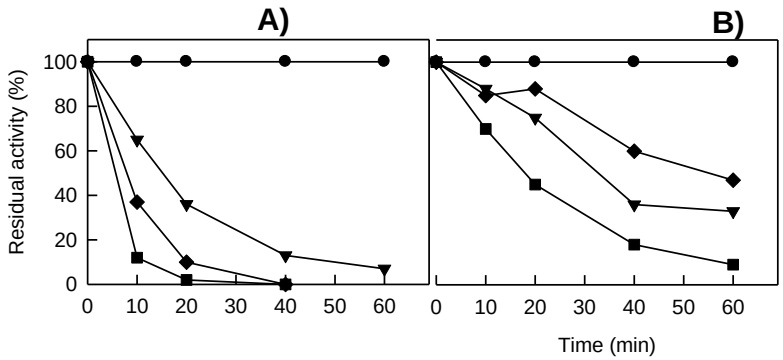

Fig. 4: Effect commercial detergents on protease stability on AL-20 protease at 60ºC (●), *subtlisin at 50ºC* (■), *Proteinase K at 60ºC* (▼) *and endogenous enzyme at 50ºC* (♦) *in commercial detergents Areal* (*A) and Via (B).*

Other alkaliphilic bacteria producing alkaline proteases using feather were a subject of one MSc thesis and are available for scale up (Teka 2007). Two other MSc thesis research were conducted on the study of alkaline proteases from two new alkaliphilic bacteria (*Bacillus sp* and *Vibrio* sp) producing alkaline proteases. Both of these enzymes were tested for dehairing application of skin and hide in the absence or presence of less than half the normal concentration of dehairing chemicals. As shown in Fig. 5, in the absence of sulfide and lime both enzymes removed the hair from the root (Haile, 2009; Seid, 2011). In the process of leather tanning most of the protein and sulfide responsible for the bad odor are released during the dehairing process. Enzymatic dehairing could therefore help to avoid or reduce the amount of sulfide and soluble keratin released in the wastewater.

What is interesting is that protease R-11 is produced using hair recovered in the process of tanning. Thus the enzyme is used to remove hair from skin and hide from the base and hair is used to produce the enzyme. The medium for enzyme production was composed of only mineral salt solution and 2% hair. This shows that the production cost of the enzyme can be significantly reduced making its use highly competitive

Protease R-11 is also very useful for detergent application. White cloth stained with blood and egg was washed following standard procedures using surfactants or in the presence of the enzyme. As shown in Fig 6, compared to surfactants washing with enzyme removed all traces of stain. This clearly shows that the enzyme can find multiple applications in the detergent or leather industries and its production cost can be substantially lowered because of the use of a cheap substrate, hair.

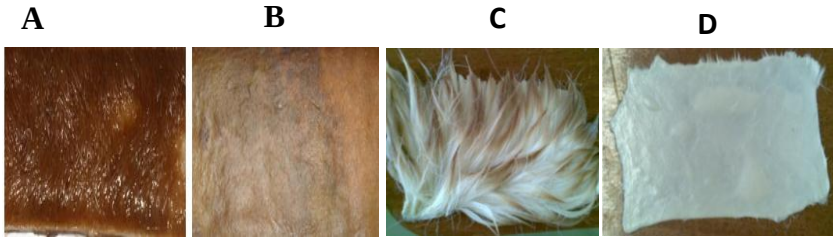

Fig. 5: Enzymatic dehairing of cow hide and sheep skin. A) Cow hide treated with buffer alone; B) Cow hide treated with alkaline protease C-45 pH 10 buffer; C) sheep skin treated with buffer alone; D) Sheep skin treated with alkaline protease R-11

Fig. 6: Evaluation washing performance of protease R-11 using blood and egg stained cotton cloth.

3.2. **Neutral proteases**

Neutral alkaline protease producing fungal strains were isolated and characterization of these enzymes was a subject of one MSc thesis research (Assefa, 2009). Both strains grow and produce the enzyme using solid state fermentation using wheat bran. The properties of these enzymes was very similar to pancreatic proteases indicating that it could serve as bating agent in leather tanning industries. Indeed, recently the enzyme was prepared in powder form and its potential for leather bating was compared with commercial enzyme preparations and a very good result was obtained. Physical properties of leather prepared using this enzyme was almost identical to leather prepared using commercial bating enzyme (Table 1). This indicates that the new enzyme can be used for bating application in the region and the way the enzyme was formulated meets the industry's standard. In addition, enzyme production was carried out in solid state fermentation using wheat bran which can lead to a significant reduction in the cost of enzyme production. This shows that the technology for the production and formulation of these bating enzymes is currently ready and scale up of this technology could be expected to have immediate impact on leather tanning industries in the region. Moreover, because of the low production cost, export of these enzyme preparations to other regions could be highly competitive.

Table 1: Physical properties of leather bated using commercial bating enzyme and new laboratory prepared enzyme

Test	Unit	Bating enzyme source	
		Commercial bating enzyme	Laboratory prepared protease (Protease BACC 480)
Tensile strength	N/mm^2	28.2	29.5
Percentage elongation	%	59.4	68.1
Tear load	N/mm	39.7	37.7
Mean tear load (parallel to the back bone)	N	28.0	24.0
Mean tear load (perpendicular to the back)	N	28.0	29.0
Average tear load	N	28.0	26.5
Distension at burst	mm	11.8	11.2

3.3. Starch degrading enzymes

In the East Africa region there is a huge potential for the production of starch based products for food and nonfood applications. For example the region grows large quantities of cassava, but to date it is only used for food following traditional processing. Although there is a potential for the production of cassava in excess of food demand in many of these countries, to date there is no industry in the region that can use it for other industrial applications. For example starch from cassava can be converted to glucose and fructose syrups, maltose syrups, maltodextrins, etc with huge potential application in the food and non-food industries.

In this connection starch degrading enzymes (amylases) are extremely important. Although starch can be hydrolyzed using acid or enzymes, at present almost all starch processing industries worldwide use enzymatic

hydrolysis. The most important enzymes for this application are thermostable amylases, glucoamylases, beta amylases, debranching enzymes, and glucose isomerases. At Addis Ababa University over the last two decades research on amylases has been carried out which resulted in the publication of several papers (Mamo *et al*. 1999; Mamo and Gessesse, 1999a and 1999b) and five graduate students did their thesis research on amylases (Mamo, 1996; Teka, 2007; Kebede, 2008; Nibret, 2009; and Damtie, 2011). Similarly at the University of Nairobi, one PhD thesis was conducted on amylases (Hashim, 2004). Many of these enzymes are potentially attractive for large scale industrial application. Recently some of these enzymes were used to hydrolyze enset starch for the production of glucose and maltose syrups (Gessesse A, unpublished data).

3.4. Alkaline and thermostable xylanases

In recent years alkaline and thermostable xylanases are becoming very important as bio-bleaching agents in the pulp and paper industry. In the kraft process of paper making wood chips are cooked at around 160°C in an alkaline medium. This helps to remove 90 to 95% of the lignin. However, the remaining lignin is highly modified and imparts a dark brown color to the pulp. To produce white paper of acceptable quality a series of chlorine based bleaching operation are carried out. Thus the residual lignin is converted to chlorinated organic compounds and washed out from the pulp. Although chlorine based bleaching is very effective in removing the residual lignin it comes with a heavy price on the environment. Chlorinated organic compounds are known to be toxic, carcinogenic, and some not biodegradable. As a result in many countries stringent environmental regulations are put in place to limit the amount of chlorinated organic compounds released. Enzyme assisted bleaching (bio-bleaching) using xylanases have been shown to lead to a significant reduction in the amount of chlorinated organic compounds.

For bio-bleaching, xylanases with optimum activity and stability at alkaline pH and high temperature are highly preferred. However, most xylanases reported from many laboratories are optimally active in the neutral to acidic pH range. In our laboratory different microbial strains producing xylanases active and stable at alkaline pH and high temperature were reported (Gessesse, 1998; Gessesse and Mamo, 1999; Yihun 2007). Some of these enzymes are produced using solid state fermentation (Gessesse and Mamo, 1999; Yihun, 2007) with very high productivity. If the production process is scaled up and the enzymes are stabilized and properly formulated, many of these enzyme could be useful for pulp and paper industries in the region and could also compete in the global industrial enzyme market.

3.5. Cellulases, and lacasses

In addition to xylanases, cellulases are also becoming extremely useful in the paper industry. For example addition of cellulases and xylanases improve the rate of drainage of recycled fiber. As shown in Fig. 3 above cellulases and lacasses are also very important in the textile industry. In our laboratory several fungal species producing cellulases and lacases were isolated and the enzymes characterized (Tewelde, 2011). Many of these organisms grow using bagass in solid state culture and all of them produce large quantities of the enzymes.

3.6. Xylanases and phytases as animal feed additives

Use of enzymes as animal feed additive is one of the fastest growing market for industrial enzymes. The most important enzymes for this application are xylanases and phytases which are added to the diet of monogastric animals. Several studies showed that non starch polysaccharides (NSP) pose serious problem in nutrient absorption and growth performance of chicken fed with wheat and barley because of its high pentostan content, the main component being arabinoxylan. Addition of xylanases helps to degrade the arabinoxylan and greatly improve growth and feed utilization efficiency. To be used as animal feed additives, xylanases need to be active and stable in the acidic to neutral range and stable in the presence of proteolytic enzymes in the gut. Earlier xylanases with enormous potential for animal feed application were isolated from two higher fungal species (mushroom types) (Tulu, 2007; Jemaneh, 2008). In both cases the enzymes were produced using solid state cultivation with very high enzyme yield. After maximum enzyme production is achieved, the whole solid state culture was dried and powdered (together with the fungal mycelium) and mixed with the feed. However, the palatability and safety of this preparation is not yet studied. Provided this formulation is safe and acceptable by the animal, it could greatly reduce enzyme production cost. Moreover, inclusion of the fungal mycelium in the feed could help to enrich the protein content of the feed. Other organisms of potential interest as animal feed additives are phytases producing fungal strains recently isolated in our laboratories. At present these enzymes are being characterized and cultivation conditions optimized.

3.7. Enzymatic hydrolysis of protein wastes

Large quantities of plant and animal protein are released annually as waste. These proteins, after enzymatic hydrolysis can be used for a variety application, such as animal feed and food supplements, microbiological media, cosmetics, leather tanning supplements, etc. One such waste is keratin that is released in the form of feather and hair from poultry and leather tanning industries, respectively. Over 90% of keratin is protein, but it is highly crystalline and cannot be digested by most enzymes. But some microorganisms are capable of hydrolyzing keratin releasing soluble proteins and amino acids that can be used for a number of applications. In our laboratory several keratin degrading microorganisms were isolated (Gessesse *et al.* 2003; Teka, 2007; Seid, 2011; Simachew *et al*. unpublished data). When one of these organisms, strain R-11, was grown in minerals salt solution supplemented with 10 g/l keratin, it released 3 g/l soluble protein indicating that 30% of the keratin is now available as soluble protein. After separation of cells and other insoluble particles the concentrated cell free supernatant can be used as animal feed supplement. If the organisms are not toxic, another option would be to dry everything and use it as animal feed supplement.

Another study carried out in our laboratories was enzymatic hydrolysis of plant and animal proteins for the production of microbiological media. At present media used for clinical and research laboratories is imported with the expenditure of foreign currency. Cost is often a limiting factor for routine use of culture for diagnostic purposes in many clinical labs. Similarly, research labs often sit idle for lack of appropriate media to carry out research. Recently in our lab protein isolated from brebra seed meal, an oil rich legume, and peptone was prepared after enzymatic hydrolysis. The result was extremely encouraging where the peptone prepared in our lab performed better than commercial peptones (Fig. 7) (Andualem, 2010; Andualem and Gessesse, 2011). In another study in our lab one MSc study was conducted where nug (*Gizotia abyssinica*) meal was enzymatically hydrolyzed and used for the growth of different test organisms. All organisms grew extremely well in these products indicating that hydrolyzates prepared from locally available protein wastes could be useful as microbiological media.

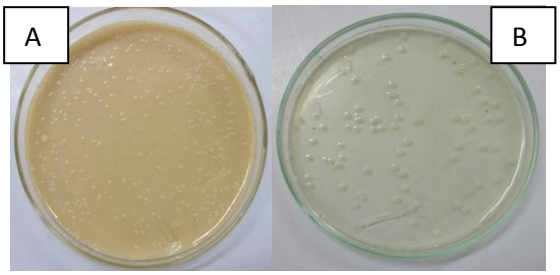

Fig. 7: Growth of Shigella using laboratory prepared brebra peptone (A) and commercial peptone (B)

Other protein wastes of interesting potential application in different industrial processes include those released by the leather tanning, meat, poultry, and fish processing industries. For example enzymatic hydrolysis of fish waste is very useful as a milk replacement for growing calves. Similarly protein hydrolyzates from trimmings in the leather industry could find a number of applications and at the same time reduce the impact of such waste on the environment.

4. Current market for industrial enzymes

At present different industries in the region import substantial quantities of enzymes for use in leather tanning, textile, and brewing industries. Other industries shy away from importing enzymes because of cost factor. For example in Ethiopia more than 150,000 kg of bating enzyme is imported annually with a cost of US$ 900 - 1000 thousand (Leather Development Institute, pers. Comm.). Tanzania is another country with high potential for leather tanning where the annual cost of bating enzymes is estimated to be in the range of US$ 500 – 800 per annum. Thus, the existing market for bating enzymes in the East African region (Ethiopia, Tanzania, Kenya, Rwanda, Sudan, and Uganda) is estimated to be about US$ 3.8 million per annum.

To date no tannery in the region is using enzymes for soaking and dehairing applications, mainly because of cost and lack of availability of such enzymes with affordable price. If enzymes are used for these processes substantial quantity of chemicals imported could be replaced thus saving foreign currency expenditure.

Although the region has huge potential for the development of starch hydrolysis products, to date no one uses this process for value addition. For example cassava, sweet potato, enset, and other root crops widely grown in the region can be used as starting material for the production starch hydrolysate. One application for this is replacement of part malt used for brweing. Currently one ton of malt costs about US$1000. Barley produced in the region does not meet the demand for malt. For example in Ethiopia more than half of the malt is imported where more than 40,000 ton of malt with a cost of about US$ 40 million is imported every year. In our earlier study we showed that up to 25 -30% of the malt can be replaced by starch hydrolysate. If a quarter of this is replaced by enzyme hydrolysate the country could save at least US$ 10 million a year. If we consider other countries in the region the annual foreign currency saving is estimated to be more than US$ 50 million.

Amylases are also imported to supplement malt in breweries, for textile desizing, etc. However, current usage for these enzymes is low. With availability of cheap and reliable enzyme supply these and a number of many other industries (such as animal feed, pulp and paper, food, etc) are expected to use enzymes in their process. This shows that the region has significant market for industrial enzymes and new application areas are expected to open.

In the process of enzyme production up to 40% of the production cost is always accounted for by the growth substrate. The enzymes considered in this study grow using very cheap substrates such as wheat bran for SSF or hair and feather for protease producing strains grown using SmF. For example if glucose, peptone, and yeast extract are to be used for our 300 l fermenter the cost of the substrate (based on current local price) is estimated to be US$ 350 per batch. If hair or wheat bran is used the cost of the growth substrate will be less than US$50 per batch. However, cost effectiveness a certain enzyme does also depend on the level of enzyme production. Therefore, due attention has been given in selecting high yielding strains and in the optimization of the fermentation condition.

5. OBJECTIVES OF THE STUDY

The main objectives of this study are to:

5.1. Scale up production of different industrial enzymes discovered so far using solid state fermentation and submerged fermentation
5.2. Scale up downstream processing and optimize methods for enzyme stabilization
5.3. Collaborate with local industries in the region to evaluate different enzymes under actual industrial application condition

6. EXPECTED OUTPUTS AND OUTCOMES

Production of industrial enzymes locally is expected to have several benefits. First, because of the availability of unique microbial diversity, globally competitive enzyme producers are expected to emerge in the region. This in addition to creating job and generate income, will encourage development in other areas of industrial biotechnology. Second, local production of industrial enzymes will help many industries in the region to be globally competitive and lead to significant reduction in environmental pollution. Over all, availability of industrial enzymes locally with sufficient quantity and reasonably cheap will have significant economic and environmental benefit to the region.

7. STUDY PLAN

7.1. Enzymes to be considered in the study

Three enzyme types of (two proteases, two amylases, and two xylanases, total six enzymes) will used for large scale production and tested for 8 different application as shown in Table 2. Sufficient amount of each enzyme will be produced, formulated in powder or liquid form, and supplied to different industries for evaluation. The

different industries that will participate in the study include leather tanning, textile, pulp and paper, animal feed processing, starch, and detergent industries. The enzymes will use both submerged and solid state fermentation.

Table 2: Enzymes chosen for large scale production and their application

No	Enzyme	Mode of production	Application to be tested for:
1	Neutral protease	Solid state fermentation	Leather bating
			Protein hydrolysis for microbiological media
2	Alkaline protease	Submerged fermentation	Leather soaking and dehairing
			Detergent additive
3	α-amylase	Submerged fermentation	Starch hydrolysis for food application
			Textile desizing
4	Glucoamylase	Solid state fermentation	Starch hydrolysis
5	Alkaline xylanase	Solid state fermentation	Pulp bleaching
6	Neutral xylanse	Solid state fermentation	Animal feed

7.2. Overall process for enzyme production and downstream processing

The process that will be employed for the production and downstream processing of all the enzymes that will be considered in this study is shown in Fig. 8. Both submerged fermentation (SmF) and solid state fermentation (SSF) will be used for enzyme production. All the enzymes considered in this study are extracellular. Thus after the culture reach stationary phase enzymes will be separated from the growth substrate and concentrated.

For submerged fermentation a bioreactor with 300 l capacity will be used. With such large volume the requirement for nitrogen and carbon sources (such as peptone, yeast extract, and glucose) could be enormous. Calculation based on the current price of such media components in Ethiopia showed that the cost of carbon and nitrogen sources could be as high as US$ 350 per batch of culture. To reduce cost animal and plant protease (from legumes) will be hydrolyzed in a stirred reactor, dried using a spray drier and used for the growth of the different organisms. At the stationary phase the culture will be pumped to a continuous centrifuge and cells will be separated from the culture supernatant.

For solid state fermentation a reactor with a total capacity of holding 100 – 200 kg moldy bran (or about 50 – 100 kg dry bran basis) will be used. After maximum enzyme production is reached, depending on the enzyme, the moldy bran will be suspended in appropriate volume of water, buffer or 10 -20% ethanol solution (based on the enzyme type) and continuously stirred for one to 4 hours. After initial filtration using large sieves, the liquid will be pumped to a continuous centrifuge. The solid substrate (which is rich with fungal mycelia) could be used for animal feed. If it is not palatable or if it has any toxin, the whole biomass will be used to generate biogas. Similarly cells and particulate matter separated by centrifugation will be mixed with water and pumped to the biogas plant to decompose.

The cell free supernatant will be pumped to a stainless steel reactor and precipitated by adding appropriate volume of solvents (ethanol or acetone). The mixture will be pumped to a centrifuge and precipitated protein recovered. After removing residual solvents, the protein will be formulated as powder or liquid product (with appropriate stabilizers added) and distributed to different industries.

7.3. Scale up of SSF and SmF for large scale enzyme production

Earlier different organisms producing neutral proteases (Assefa, 2008; Gessesse *et al*, unpublied data), neutral xylanases (Degefu, 2007; Zeleke, 2008), alkaline xylanases (Gessesse and Mamo, 1999; Yihun, 2007)

glucomylases (Mamo and Gessesse, 1999; Teka, 2007) and alpha amylase (Kebede, 2008; Nibret, 2009) were grown using solid state fermentation at a laboratory scale. In this study the process will be scaled up at Addis Ababa University and at the University of Nairobi. Data obtained at this stage will be used for large scale production at the pilot plant. The fermenter will be constructed from stainless steel and the process for medium sterilization, aeration, cooling, and maintenance of humidity will be scaled up. Harvesting will be manual and downstream processing will be carried out as outlined in Fig. 8.

Other microorganisms producing such enzymes as alkaline proteases (Gessesse et al. 2003; Haile, 2009; Seid, 2011) alpha-amylases (Mamo and Gessesse, 1999; Damtie, 2011) and xylanases (Sitotaw, unpublished data) can only grow using submerged culture. Scale up studies for submerged fermentation shall be carried out using 5 l fermenter (New Brunswik) currently available in our laboratories.

6.3. Evaluation of the industrial application of enzymes under application condition

All enzymes produced will be tested for different applications under application condition. For enzymes used in the leather industry initial studies will be carried out at the Leather Development Institute in Ethiopia where they have a battery of different sized experimental units. Once the process is optimized it will be tested under factory condition. Finally the enzymes will be given to different leather industries in the region to test under routine application conditions. To build confidence enzymes found acceptable by the leather industries will be distributed free so that they will be willing to buy it in the future.

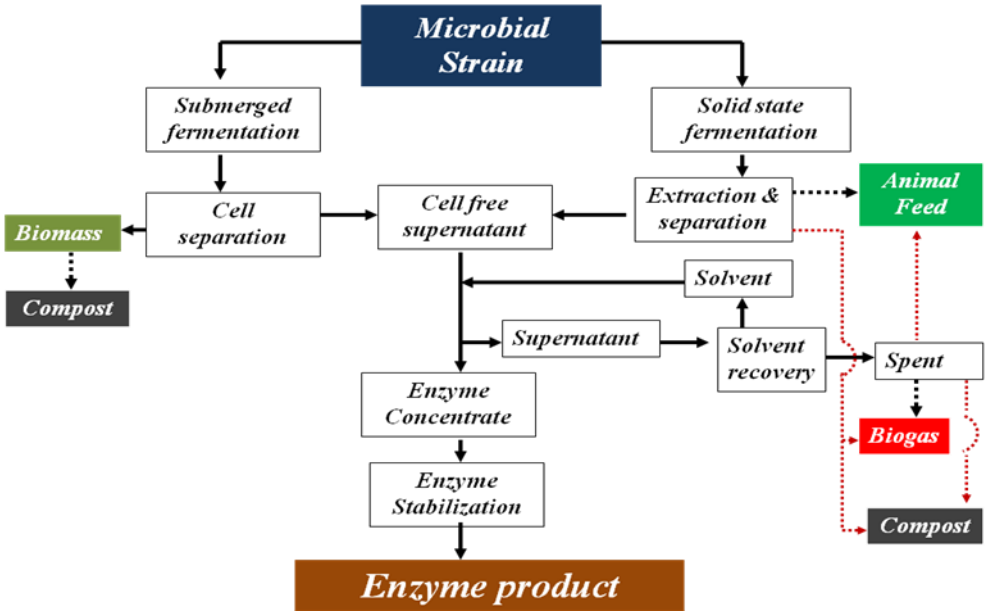

Fig. 8: Process flow chart for enzyme production

In the textile industry attention will be focused to the production and use of thermostable amylases used for desizing use. Currently industries in the region are not using other enzymes in their process. This could help to creat a working relationship between our team and the textile industries in the region. In the long term therefore, these industries could develop the confidence to use other enzymes which are now considered essential. Should this happen, the process developed in this study can be used to produce other enzymes for the textile industry, such as pectinases, lacasses, and cellulases.

In developed countries more than 90% of detergents contain alkaline proteases. In our previous study alkaline proteases active and stable in the presence of commercial detergents were developed. In this study alkaline protease will be produced and tested for detergent use. This test will conducted in collaboration with Bekas Chemicals Plc, Ethiopia, a detergent manufacturing company. Bekas has already been in search of a source for

enzymes to include in its products. If the enzyme containing detergent find consumer acceptance the company agreed to start full scale production of enzyme containing detergents.

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Potentially interesting xylanases for biobleaching application in the pulp and paper industry have been isolated at Addis Ababa University and the University of Nairobi. Many of the countries in the region import bleached pulp for paper production. But, in Kenya there is a pulp factory which uses traditional processes of bleaching. Therefore, testing the potential usefulness of the enzymes will be carried out at the University of Nairobi in collaboration with the pulp and paper factory. Should the result be encouraging, after settling IP issues, enzyme samples will be further tested at paper and pulp factories in Sweden in collaboration with the Lund University.

For starch hydrolysis initially we shall team up with breweries in the region where we shall supply amylases to supplement malt so that they can use more adjunct and replace up to 25% of imported malt. Already an agreement has been reached with two breweries to test our enzymes and more industries are expected to participate. Starch hydrolysate from local crops (cassava, enset, sweet potato, etc) will be prepared using amylase and glucoamylase enzymes and prototype products (such as candies, jams, etc) will be prepared and demonstrated to interested parties.

For animal feed application, initially neutral xylanase produced by *Xylaria* sp. and other fungal species will be tested as additive for poultry and swine feed. This study will be carried out at the University of Dar es Salaam in collaboration with SAAFI, and at the National University of Rwanda. Later on after the system is developed and if there is time, phytase will be produced and tested as feed additive for fish and Swine at the University of Nairobi and the National University of Rwanda.

Finally proteases will be used to hydrolyze animal and plant proteins. The reaction will be carried out in a thermostated reaction vessel fitted with a stirrer. After the reaction reach the desired degree of hydrolysis it will be spry dried and used as peptone for the growth of different bacteria. Suitability of these products for the growth of a range of microorganisms (including our own strains) will be carried out at the University of Dar es Salaam and at Addis Ababa University.

7. COMMERCIALIZATION

Because of the presence of unique natural environments found nowhere else in the world, such as alkaline soda lakes, alkaline and neutral hot springs, Eastern Africa is an ideal place for the development of novel enzymes. For example Genecor annually earns more than US$600 million from a single enzyme it isolated from a Kenyan soda lake. Thus to make full use of this resource it is important that enzyme producing companies be established in the region. Through sustained support by Sida/SAREC and other donors in the region on industrial biotechnology the region acquired modest facility and trained manpower. What is now lacking is translating this capacity to utilize the available biological resource for biotechnological application. At the end of this study we envisage a company capable of standing by its own be established by a joint effort of the researchers involved, the participating institutions, and the private sector in the region.

The path that will be followed in this study for the commercialization of industrial enzymes is outlined in Fig. 9 below. From our previous studies enzymes of significant potential application were discovered. But most studies to date were laboratory scale and there is a need to scale up enzyme production at pilot scale. Therefore, the enzymes will be produced in large quantities and distributed to different industries in the region. To minimize cost at first each enzyme will be tested at one or two factories. If the result is acceptable more factories will be given free samples in exchange for the data. The performance of each of the enzymes will be collected, analyzed, and compiled. For enzyme products and/or processes that are unique a patent application will be filed.

To attract the attention of local inventors or to raise capital a workshop will be organized and the private sector in the region will be invited. Together with local private sector (or as alternative) international companies with expertise and interest in the area of enzyme production or industrial biotechnology will be invited and the possibility of initiating a joint company shall be discussed.

Some of the participating institutions have already started planning on the establishment of support facility for innovation. For example Addis Ababa University is currently in the planning phase to initiate institutional innovation incubation centers. The plan is to support innovations in research labs to start pilot scale productions at incubation centers and then encourage establishment of spin of companies. Enzyme technology is one of the areas selected for support and it is expected to ultimately lead to the establishment of a separate spin off company. This approach, in addition to establishing new companies, is expected to inspire young researchers in the region by showing them the possibility of changing their research results into financial gain.

Since June/ July 2011 top management at Addis Ababa University decided to establish an Institute of Biotechnology from the present Biotechnology Department/Program Unit. One of the four Departments in the new institute will be Technology Incubation and Transfer. The establishment of the Institute will be finalized in early 2012. Therefore, Addis Ababa University is committing more resource in support of innovation and technology incubation in the area of Biotechnology. A one story building with three wings (located adjacent to the present department of Biotechnology building) has already been earmarked for the incubation.

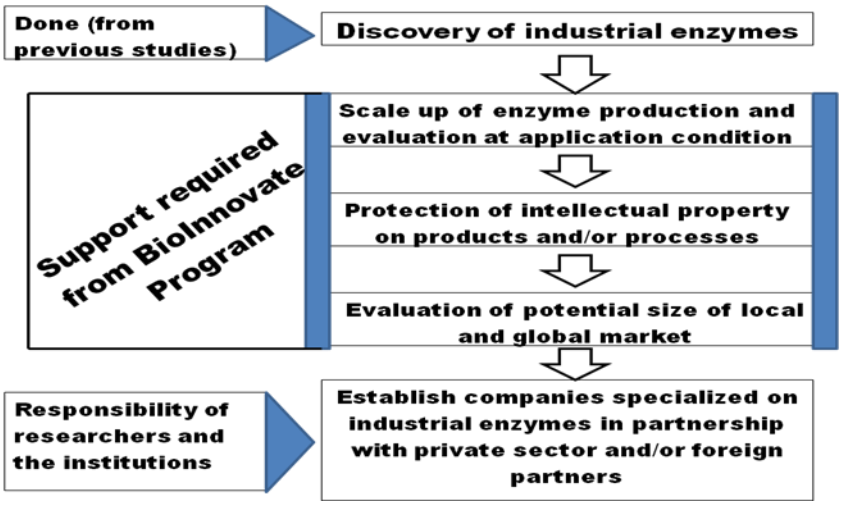

Fig. 9: Path for the commercialization of industrial enzymes

Because of support from the Addis Ababa University and because it is costly to establish large scale production facilities in all the institutions, the pilot plant will be placed at Addis Ababa University. However, strains used for large scale production and lab scale optimization of enzyme production will be arranged in each participating labs. Participants from each institution shall use the facility equally and produce the enzymes of their choice at the facility. The blue print and all other relevant data will be freely available to all participating researchers.

8. INTELLECTUAL PROPERTY (IPR) ISSUES

In this study appropriate rules and regulations to address IPR issues will be taken. Products and processes developed in this study shall be patent protected. Ownership of products developed before the start of this project shall be owned by the lab which developed it. New products developed in this project in collaboration between two or more participants will be shared by the participants. Each time microbial strains are transferred from one lab to another, appropriate material transfer agreements shall be signed. The project team shall seek further support on policy issues from projects specifically addressing such issues in the region.

9. INSTITUTIONAL SUPPORT

In this project, researchers from four countries in Eastern Africa (Ethiopia, Kenya, Rwanda, and Tanzania) will be involved. The researchers are drawn from public universities and private firms. Four private firms

from Ethiopia and Tanzania will take part in the project. In the course of the study other private firms will also join the team. In addition researchers from the region two highly experienced and renowned scientists from Sweden and India will take part in the study.

The Leather and Leather products institute in Ethiopia will participate in all research involving the leather industry. A large proportion of the cost in testing several enzymes during the study will be covered by this institution. In addition three senior members and two technical staff, fully paid by the institute will work in this project. From Addis Ababa University, University of Nairobi, University of Dar es Salaam, and National University of Rwanda, the respective Universities shall cover costs associated with salaries of researchers and support staff and provide lab space with all the required utilities. The private industries will cover cost of technical personnel involved in the project and also cover part of the cost associated with testing products.

10. COMPOSITION OF THE TEAM

10.1.Dr. Amare Gessesse, *PI, Biotechnology Program, Addis Ababa University, Ethiopia*

Dr. Amare Gessesse was trained in the area of Biotechnology and has research experience on industrial enzymes spanning nearly two decades. He started working on enzymes in 1993 as a PhD student under the supervision of Profs. Bo Mattiasson, and R-Hatti-Kaul, Department of Biotechnology, Lund University, Sweden and Prof. B. A. Gashe, Addis Ababa University, Ethiopia as part of a Sida/SAREC supported project. During this period he worked in a Sandwich mode both in Sweden and in Ethiopia. After finishing his PhD study, in 1999 he moved to the Department of Biotechnology, Aalborg University, Denmark and participated in different research projects involving microbial enzymes. In Denmark he worked on different projects in collaboration with industries using enzymes or producing enzymes. In 2005 he returned back to Ethiopia and joined the then Department of Biology, Addis Ababa University. At present Dr. Gessesse is a member of the Biotechnology Program Unit at Addis Ababa University. Over the years he published many research reports on reputed international journals and supervised several students. Starting from 2005 he supervised 40 MSc and PhD students, where half of them work on microbial enzymes of industrial importance. Currently he is PI of a project entitled "*Biotechnology and microbial diversity of Ethiopian soda lakes"* supported by the Norwegian funding agency NUFU with a budget of 5.55 million NOK. Many of the microbial strains proposed in this study were isolated through this project. He is also PI for a BIOEARN project entitled *Enset agroprocessing* and another Project on Bioenergy supported by DelPHE, UK. Over the years Dr. Gessesse worked on novel proteases, xylanases, amylases, and lipases. At present he has research collaboration with York University, UK; Bergen University, Norway; BecA, Kenya and; The University of the Western Cape, South Africa.

10.2. Prof. Francis Mulaa, *Co –PI, Department of Biochemistry, University of Nairobi, Kenya*

Professor Mulaa is a biochemist by training and was involved in microbial biotechnology research for over two decades. Over the years he published more than 30 research articles and book chapters. Prof. Mulaa has been principal investigator and Co-PI for projects supported by Sida/SAREC, IFS, BIOEARN, European Union, Gates Foundations, UNESCO, etc. He supervised six PhD students and about 20 MSc students in different areas of biotechnology. In relation to industrial enzymes his laboratory has been working on amylases, pectinases, and lipases. Currently Professor Mulaa has research collaboration with researchers in UK, Germany, Ethiopia, and Sweden.

10.3. Dr. Sylvester Leonard Lyantagaye, *CO-PI, Department of Molecular Biology and Biotechnology, University of Dar es Salaam, Tanzania*

Dr. Lyantagaye got his PhD in 2005 from the University of the Western Cape currently working as a biochemist at the University of Dar es Salaam**.** Over the years he published about a dozen scientific articles in reputable international and local journals. He has a broad research interest. Recently Dr. Lyantagaye got interested to work on microbial enzymes of potential interest for industrial and agricultural applications, especially on amylases, xylanases, and pytases.

10.4.Dr. Laetitia Nyina-wamwiza*, CO-PI, National University of Rwanda, Rwanda*

Dr. Laetitia Nyina-wamwiza works jointly (50:50) at the Department of Animal production, Faculty of Agriculture and Department of Biology, Faculty of Science at the National University of Rwanda. She did her PhD in Biology at the University of Namur, Belgium in 2007. Currently Dr. Laetitia is involved in different research projects at the Faculties of Science and Agriculture.

10.5.Professor Bo Mattiasson, Department of Biotechnology, Lund University, Sweden

Professor Mattiasson is a highly experienced scientist considered by many as one of the fathers of European Biotechnology. Professor Mattiasson established the Department of Biotechnology at Lund University in the mid 1980s and served as head of the Department for a long time. Over the years he supervised several PhD students and published several hundred scientific papers and dozens of books. Professor Mattiasson made significant contribution in establishing Biotechnology Capacity in Africa. Over the years he supervised many PhD students from Ethiopia, Kenya, and Zembabwe. The PI of this proposal, Dr. Gessesse was one of his students

10.6.Professor Ashok Pandey, Centre for Biofuels & Biotechnology Division, National Institute for Interdisciplinary Science and Technology, Trivandrum-695 019, India

Professor Pandey is a renowned scientist in biotechnology worldwide. He is world authority in solid state fermentation. He has published over 800 scientific papers, over 12 patents, and authored several books. Today he has the highest number of publications in solid state fermentation than anyone in the world. India is one of the most successful countries in the world in using solid state fermentation for biotechnological applications and Prof. Pandey is the main driving force behind this success. Most publications and patens on scale up of solid state fermentation in the literature were done under his supervision. In this study Prof. Pandey will share his vast experience in India and assist the work of the East African teams on scale up of solid state fermentation for large scale enzyme production.

10.7.Leather and Leather products institute, Addis Ababa, Ethiopia

The Leather and Leather products institute is a semi private institution established to assist development of the leather sector in Ethiopia. The Institute has facility for all stages of leather tanning (from experimental to production scale machinery) and a state of the art laboratory facility for leather and lather products analysis.. In addition to helping Ethiopian leather sector, the Institute is providing different support for leather industries in Eastern Africa. Recently it signed a US $30 million contract with the government of Sudan to give training and other supports for the leather sector in Sudan. In this study the institute will play a leading role in testing all enzymes intended for application in leather industries. One of the aims of the institute is to help replace most of the inputs used by leather industries locally. Thus enzymes are one of the most important inputs that the Institute put an effort on. Thus a team of three experts and two technicians will be assigned for this project.

10.8.Bekas Chemicals Plc, Addis Ababa, Ethiopia

Bekas chemicals Plc is engaged in the production of detergents and cosmetics. In this project Bekas will play a key role in testing some of the enzymes as detergent additives. Bekas is also interested to evaluate protein hydrolyzates for the production of shampoo and other personal hygiene products. The General Manager, a chemist by profession and two other experts from this company will work with the rest of the project team. Salary for these experts and some of the costs for this study will be covered by the company itself.

10.9.Modjo Tannery Plc

Modjo tannery plc is a private company specialized in leather tanning. In this study Modjo Tannery will participate in testing enzymes for bating, soaking, and deharing applications.

10.10. Sumbawanga Agriculture and Animal Food Industry (SAAFI), Tanzania

SAAFI is private company in Tanzania involved in the production of animal feed. In this study it will play an active role in testing the different enzymes intended as animal feed supplements.

10.11. Yalemzewd Molla*, Insitute of development studies and Department of Economics, Addis Ababa University*

Mr. Molla is an economist by training and currently he is working at the Institute of Development studies.

10.12. Other private sector partners

Currently search for other private sector partners in Kenya, Uganda, Rwanda, and Tanzania is under way. The target is to bring as many relevant privates firms as possible so that they can participate in the study.

11. EXPERIENCE AND ROLE OF EACH TEAM MEMBER IN THE STUDY

Although there is a huge potential for the growth of industrial biotechnology, to date there are only few trained people in this area in the region. Ethiopia and Kenya, because of earlier support from Sida/SAREC, had a chance to develop human capacity in this field. Therefore, there is noticeable difference in the level of experience among team members. However, we strongly believe that people trained in related fields, working in collaboration with experienced team members could play crucial roles in laying the foundation for the growth of industrial biotechnology in the region. Therefore, in this team some members are experienced biochemists, others are biologists interested in using enzyme products for different application. Team members share activities based on their experience. However, to help develop capacity each lab will have to monitor activity and stability of the different enzymes they are working on. In addition each team member is expected assist industries in the region in matters related to enzyme usage. There is a strong belief that industries in the region will be more confident to use industrial enzymes in their process if they get support and advice locally.

The following table shows the project team members and their role in the project. In addition, at the pilot plant one chemical engineer with MSc degree in chemical/biochemical engineering will be employed and run routine operations.

Team member	Activity in each budget year		
	Year 1	**Year**	**Year 3**
Dr. Amare Gessesse AAU, Ethiopia	• Purchase all equipment for the pilot plant, commission, and purchase growth substrates • Employ a chemical engineer and technical assistant • Study SmF and SSF scale up • Renovate incubation centre rooms • Purchase glassware, reagents and & consumables required for the study • Visit collaborating labs in the region, prepare material transfer agreements, and give strains to collaborators • Organize a project meeting in Addis • Coordinate the overall activity of the project	• Purchase consumables and growth substrates for pilot plant • Prepare protein hydrolysate test its use for microbial media • Send formulated enzymes to team members • Test enzymes for bating and dehairing, and detergent application • Visit regional team members • Visit the lab in Lund • Attend one	• Purchase required supplies for pilot plant and keep it operational • Send formulated enzymes to team members • Test enzymes for leather and detergent application • Test amylases for starch hydrolysis in the textile & brewing industry • Prepare protein hydrolysate for microbial media • Organize workshop

	• Visit different local industries in Ethiopia, gather data on their enzyme usage, and create a working relation • Visit International collaborators lab (Lund and India) discuss about pilot plant and share experience • Write annual report	conference and present results • Coordinate overall activities of the project • Prepare annual report • Write annual report	and project meeting • Regional travel • Attend one intl conference and present a paper • Prepare final report
Prof. Francis Mulaa, UoN, Kenya	• Purchase required laboratory equipment and reagents • Study scale up of SSF and SmF for selected strains • Employ a technical assistant • Organize the first project meeting in Nairobi • Travel to different industries in Kenya and study their enzyme requirement and create a working relation • Travel to Addis for pilot plant commissioning	• Purchase supplies, consumables, and small laboratory equipment • Supervise technical assistant • Evaluate xylanases for pulp biobleaching and employ labor for handling • Test enzymes as animal feed additives • Local travel to different industries for evaluation and coordinate the activity in Kenya • Attend a project meeting • Attend one international conference and present a paper	• Purchase supplies for the lab • Supervise technical assistant • Evaluate xylanases for pulp biobleaching and employ labor for handling • Test enzymes as animal feed additives • Local travel to different industry sites and process optimization • Attend one international conference and present a paper
Dr. Sylvester Lyantagaye, UDSM, Tanzania	• Purchase lab equipment and organize the lab for enzyme analysis • Travel to different industries in Tanzania and study their enzyme requirements & create a good working environment • Attend project meetings in Addis and Nairobi	• Purchase reagents and keep the lab running • Evaluate enzymes for tannery application in Tanzania and employ temporary labor • Team up with local breweries for starch hydrolysis and evaluation of its potential • Collaborate with SAAFI to test xylanases for feed application • Test suitability of protein hydrolysates for microbial media preparation • Travel to different industries in Tanzania	• Purchase reagents and keep the lab running • Evaluate enzymes for tannery application in Tanzania and employ temporary labor • Team up with local breweries for starch hydrolysis and evolution of its potential • Collaborate with SAAFI to test xylanases for feed application • Test suitability of protein hydrolysates as microbial media

		and coordinate evaluation studies • Attend project meeting • Attend one international conference and present paper	• Organize project meeting • Extensively travel to different industries and coordinate enzyme evaluation in Tanzania • Travel to Addis for workshop and final project meeting • Attend one international conference and present paper
Dr. Laetitia Nyina-wamwiza NUR, Rwanda	• Purchase lab equipment and organize the lab for enzyme analysis • Travel to different industries in Rwanda and study enzyme requirement and create a good working relation • Attend two project meetings	• Purchase lab supplies and run the laboratory • Evaluation of enzymes for animal feed use (including as fish feed) • Organize project meeting • Visit laboratory in Nairobi • Attend one international conference and present paper	• Purchase lab supplies • Evaluation of enzymes for animal feed use • Attend project meeting • Attend one international conference and present paper
Prof Bo Mattiasson Lund University, Sweden	• Attend project meetings and discuss with team members • Assist in lab analysis (avail expertise and facility)	• Attend project meetings and discuss with team members • Assist in lab analysis (avail expertise and facility)	• Attend project meetings and discuss with team members
Prof Ashok Pandey, India	• Attend project meetings and discuss with team members	• Attend project meetings and discuss with team members	• Attend project meetings and discuss with team members
Leather Industry Development Institute, Ethiopia		• Test enzymes for batting, dehairing, and soaking application	• Test enzymes for batting, dehairing, and soaking application
Bekas Chemicals Plc, Ethiopia		• Test alkaline proteases for detergent use	• Test alkaline proteases for detergent use
Modjo Tannery Plc		• Test enzymes for	• Test enzymes for

		batting, dehairing, and soaking application at industrial scale	batting, dehairing, and soaking application at industrial scale
Sumbawanga Agriculture and Animal Food Industry (SAAFI), Tanzania		• Test xylanases and phytases as animal feed additive	• Test xylanases and phytases as animal feed additive
Yalemzewd Molla		• Advice and plan on marketing and commercialization of industrial enzymes	• Advice and plan on marketing and commercialization of industrial enzymes

12. PROJECT COORDINATION AND CONSORTIUM MEETINGS

To share experience and discuss results, regular meetings of all project personnel will be conducted. Tentative meeting schedules are shown in Table 4 below. First a launching meeting will be held in Nairobi at the BioInnovate head quarters or at the University of Nairobi. In this meeting plans will be discussed in detail and information exchanged among members.

In the first half of Year 1 the pilot plant is expected to be operational. Thus in the fourth quarter of Year 1 a meeting will be organized in Addis Ababa and all project team members will present their results and challenges faced and all issues will be discussed in detail. A third meeting is scheduled to be held in Rwanda to discuss on progress and evaluate the performance of each member in relation to the planned activities.

In Year 3 there will be one meeting in Tanzania at around the second quarter to discuss on progress and plan on how to attract the private sector in the region and internationally. At the end of the project a three day workshop will be organized in Addis Ababa. A total of 75 to 80 participants from enzyme using industries (tanneries, textile industries, breweries, etc) and entrepreneurs in Tanzania, Ethiopia, Rwanda, and Kenya will be invited for the workshop. The pilot plant will be visited and results obtained from full scale application tests will be discussed. In addition the PI will visit the activities of each laboratory and discuss any problem encountered.

Table 4: Meeting schedules

Budget Year	Meeting venue (institution)					**Remark**
	Quarter	UoN, Kenya	AAU, Ethiopia	NUR, Rwanda	UoDS, Tanzania	
Year 1	1st	XX				Launching meeting at BioInnovate head office or at UoN
	2nd					
	3rd					Commissioning of the pilot plant refining of next activities in the plan
	4th		XX			
Year 2	1st					Progress report for each laboratory, identify problems and take corrective measures
	2nd			XX		
	3rd					
	4th					
Year 3	1st				XX	Progress report and discussion on future plans
	2nd					
	3rd					Workshop and exhibition of the pilot plant and conclusion of the project
	4th		XX			

13. MILESTONES AND TIME FRAME

This project is a three year project and involves construction of a scale enzyme production facility.

Table 5: Project activity plans for all partner institutions

Activity	Person Involved	Year 1		Year 2		Year 3	
		H1	H2	H1	H2	H1	H2
Activity 1: Scale up of enzyme production, stabilization, & standardization of enzyme assay conditions							
1.1 Scale up and optimization of Solid state fermentation (SSF) process	Amare Gessesse, AAU Francis Mulaa, UoN Ashok Pandey, India						
1.2 Optimize enzyme stabilization and formulation	Francis Mulaa, UoN Bo Mattiasson, Lund U Amare Gessesse, AAU						
1.3 Optimize & formulate medium for growth and enzyme production using SmF	Amare Gessesse, AAU Francis Mulaa, UoN						
1.4 Standardize enzyme assay methods and establish a functioning laboratory	S. Lyantagaye, UDS L. Nyina-wamwiza, NUR						
Activity 2: Commissioning of pilot plant, large scale enzyme production, & enzyme formulation							
2.1 Installation of solid state fermenters, centrifuge, solvent recovery facility, spray drier, & pumps	Amare Gessesse, AAU						
2.2 Large scale enzyme production using SSF & enzyme formulation	Amare Gessesse, AAU Chem Engineer, AAU						
2.3 Purchase and commissioning of liquid fermeter for SmF	Amare Gessesse, AAU						
Activity 3: Hydrolysis of plant and animal proteins and evaluation as microbiological media							
3.1 Optimize enzymatic protein hydrolysis & test the product as microbial media component	Amare Gessesse, AAU Chem Engineer, AAU						
3.2 Test protein hydrolysates as microbiological media	S. Lyantagaye, UDS Amare Gessesse, AAU						
Activity 4: Evaluation of proteases for application in the leather tanning and detergent industry							
4.1 Microbial proteases for bating application	Amare Gessesse, AAU S. Lyantagaye, UDS LIDI, Ethiopia Modjo Tannery						
4.2 Alkaline proteases for soaking	Amare Gessesse,						

and dehairing application	AAU S. Lyantagaye, UDS LIDI, Ethiopia Modjo Tannery						
4.3 Hydrolysis of solid leather industry waste and evaluation for different applications	LIDI, Ethiopia Bekas Plc, Amare Gessesse, AAU						
4.4 Application of alkaline proteases as detergent additives	Amare Gessesse, AAU Bekas Plc						
Activity 5: Evaluation of xylanases and phystases as animal feed additives							
5.1 Evaluation of xylanases and phytase for poultry & pig feed additives	S. Lyantagaye, UDS SAAFI, Tanzania Nyina-wamwiza, NUR						
5.2 Test phytases as fish feed additives	Nyina-wamwiza, NUR Francis Mulaa, UoN						
Activity 6: Application of amylolytic enzymes for starch hydrolysis							
6.1 Use of thermostable amylases for desizing in the textile industry	S. Lyantagaye, UDS Amare Gessesse, AAU						
6.2 Evaluation microbial amylases to supplement malt in breweries	S. Lyantagaye, UDS Amare Gessesse, AAU						
6.3 Starch hydrolyzates from cassava, enset, and/or cereal starch test for food application	S. Lyantagaye, UDS Amare Gessesse, AAU Francis Mulaa, UoN						
Activity 7: Evaluation of alkaline xylanases as biobleaching agents in the pulp and paper industry							
7.1 Test xylanases and cellulose for bio bleaching of pulp and deinking application	Francis Mulaa, UoN Industry partner						
Activity 8: Reporting (technical and financial)							
8.1 Preparation of annual and final reports (technical and financial)	PI and Co-PIs						

14. DETAILED DESCRIPTION OF PROJECT ACTIVITIES FOR PARTNER INSTITUTIONS

The following is a detailed description of the activities of each partner institution:

Addis Ababa University	
Activity 1: Scale up of enzyme production, stabilization, & standardization of enzyme assay conditions	
1.1	*Scale up and optimization of solid state fermentation (SSF) process*
	In preparation for large scale growth sSelected strains amedium composition for maximum enzyme yield and appropriate conditions for the cultivation will be determined and scaled up.
1.2	*Optimize enzyme stabilization and formulation*
	To avoid denaturation in the process of transportation and storage enzymes will be stabilized using different additives. Since, no single method can work equally well for all enzymes, each enzyme will be tested independently, the method optimized, and used for final product formulation.

1.3	*Optimize & formulate medium for growth and enzyme production using SmF*
	The best medium composition and optimum growth condition for growth and enzyme production using submerged fermentation will be optimized using a laboratory fermenter of 5 l capacity and used for pilot scale production.
Activity 2: Commissioning of pilot plant, large scale enzyme production, & enzyme formulation	
2.1	*Installation of pilot scale solid state fermenters, centrifuge, solvent recovery facility, spray drier, & pumps*
	Two fermenters for solid state fermentation (SSF), one for fungal and one for bacterial strains, will be purchased and installed. In addition such equipment as spray drier, continuous centrifuge, pumps, and distillation apparatus will be purchased, installed and integrated with the pilot plant.
2.2	*Large scale enzyme production using SSF & enzyme formulation*
	Large scale enzyme production will be carried out using the pilot plant facility, stabilized, and distributed to collaborators and industrial partners in the region.
2.3	*Purchase and commissioning of liquid fermeter for SmF*
	During the first quarter of the second budget year the liquid fermenter shall be purchased and installed at the pilot plant. Large scale enzyme production will be carried out. Concentrated and stabilized enzymes will then be distributed to all collaborators and industrial partners in the region.
Activity 3: Hydrolysis of plant and animal proteins and evaluation as microbiological media	
3.1	*Optimize enzymatic protein hydrolysis & test the product as microbial media component*
	Animal and plant protein sources will be hydrolyzed using enzymes produced at the pilot plant, the hydrolyzate spray dried and used for the growth of microorganisms at the pilot plant in clinical labs.
3.2	*Test protein hydrolysates as microbiological media*
	Protein hydrolyzates prepared from animal and plant proteins will be tested for the growth of different microorganisms in collaboration with research and clinical labs.
Activity 4: Evaluation of proteases for application in the leather tanning and detergent industry	
4.1	*Microbial proteases for bating application*
	Microbial proteases will be tested for bating application in collaboration with the Leather Industry Development Institute (LIDI) and Modjo Tannery. Physical properties of finished leather will be tested at LIDI's lab and enzymes giving good quality product will be tested further at a pilot scale. Enzymes giving good quality finished leather will be given to Modjo Tannery and to tanneries in Tanzania for further evaluation at application condition.
4.2	*Alkaline proteases for soaking and dehairing application*
	Performance of alkaline proteases for skin/hide dehairing will be tested in collaboration with LIDI, the best performing enzymes selected, and given to Mojo tannery and other tanneries in Tanzania for further evaluation.
4.3	*Hydrolysis of solid leather industry waste and evaluation for different applications*
	Solid waste generated at the leather industry will be hydrolyzed enzymatically and tested for a variety of applications in collaboration with Bekas Plc.
4.4	*Application of alkaline proteases as detergent additives*
	Stability and performance of some of the alkaline proteases will be carried out in collaboration with our industrial partner Bekas Plc.
Activity 6: Application of amylolytic enzymes for starch hydrolysis	
6.1	*Use of thermostable amylases for desizing in the textile industry*
	Amylases will be produced at the pilot plant, formulated as powder and/or liquid stabilized products and tested for desizing of starch size.
6.2	*Evaluation microbial amylases to supplement malt in breweries*
	Microbial amylases produced at the pilot plant will used to supplement malt amylase in breweries and the quality of beer produced tested for its physical, chemical, and oraganoleptic qualities.
6.3	*Starch hydrolyzates from cassava, enset, and/or cereal starch test for food application*

	Starch from different botanical sources will be hydrolyzed using microbial amylases and used to prepare such products as candies, jams, jellys, etc. These products demonstrated to interested investors/partners used to encourage them to invest in the area.
Activity 8: Reporting (technical and financial)	
8.1	*Preparation of technical and financial report*
	Each Co-PI will prepare annual (and biannual) reports (both financial and technical and give it to the PI). The PI will compile all reports and submit to the BioInnovate Office as required.

University of Nairobi	
Activity 1: Scale up of enzyme production, stabilization, & standardization of enzyme assay conditions	
1.1	*Scale up and optimization of solid state fermentation (SSF) process*
	For microorganisms selected from UoN, medium composition for maximum enzyme yield and appropriate conditions for cultivation using SSF will be optimized and scaled up.
1.2	*Optimize enzyme stabilization and formulation methods and test enzyme stability*
	The role of different salts and sugars to stabilize selected enzymes will be studied and optimized for large scale application
1.3	*Optimization & medium formulation for selected strains using submerged fermentation (SmF*)
	Medium composition and optimum growth conditions for the growth of microbial stains selected from the UoN will be optimized and made ready for large scale production at the pilot plant
Activity 5: Evaluation of xylanases and phystases as animal feed additives	
5.2	*Test phytases as fish feed additives*
	The potential of pytases as fish feed additive will be tested. Feed utilization efficiency and growth of fish fed with enzyme supplement will be compared with controls.
Activity 6: Application of amylolytic enzymes for starch hydrolysis	
6.3	*Evaluation microbial amylases to supplement malt in breweries*
	The potential of microbial amylases produced at the pilot plant will be tested for their potential application to supplement malt enzymes in collaboration with breweries in Kenya.
6.2	*Starch hydrolyzates from cassava, enset, and/or cereal starch test for food application*
	Starch from root crops or cereals will be enzymatically hydrolyzed and used to produce candies, jams and jellys, etc and the products demonstrated to interested people.
Activity 7: Alkaline xylanases for bio-bleaching of pulp	
7.1	*Test xylanases and cellulose for bio bleaching of pulp and deinking application*
	Alkaline and thermostable xylanases will be tested for biobleaching of pulp in collaboaration with a paper mill factory in Kenya. Further evaluation of the enzyme(s) may be carried out in Sweden at Swedish paper mills.
Activity 8: Reporting	
8.1	*Preparation of technical and financial report*
	Co-PI will prepare annual (and if required biannual) reports (both financial and technical) and give it to the PI who will compile a full project report and submit to the BioInnovate Office.

University of Dar es Salaam	
Activity 1: Scale up of enzyme production, stabilization, & standardization of enzyme assay conditions	
1.3	*Standardize enzyme assay methods and establish a functioning laboratory*
	To be able to support enzyme users in Tanzania the laboratory at UDS will be equipped with basic facility and methods for enzyme assay standardized. Thus, essential equipment will be purchased for the laboratory made functional.

Activity 3: Hydrolysis of plant and animal proteins and evaluation as microbiological media	
3.2	*Test protein hydrolysates as microbiological media component*
	Protein hydrolyzates (or peptones) prepared from animal and plant proteins at the pilot plant at AAU will be tested as component of microbiological media for the growth of different microorganisms. The product will also be distributed to different labs in Tanzania and results compiled.
Activity 4: Evaluation of proteases for application in the leather tanning and detergent industry	
4.1	*Leather bating using microbial proteases and evaluate physical properties of leather products*
	Selected microbial proteases selected for best performance for bating application will be tested in tanneries in Tanzania.
4.2	*Test alkaline proteases for soaking and dehairing application*
	Alkaline proteases with good performance as dehairing agents will be distributed to end users in Tanzania and results on its performance compiled and analyzed.
Activity 5: Evaluation of xylanases and phystases as animal feed additives	
5.1	*Evaluation of xylanases and phytase for poultry & pig feed additives*
	Xylanases and phytases will be tested as feed additive for poultry and swine. The performance of animals supplemented with these enzymes will be compared with controls receiving no enzyme supplement. This activity will be carried out in collaboration with the animal feed industry, SAAFI.
Activity 6: Application of amylolytic enzymes for starch hydrolysis	
6.1	*Thermostable amylases for desizing in the textile industry*
	Thermostable amylases will be tested for desizing application at textile industries in Tanzania. Performance data will be collected and analyzed.
6,2	*Evaluation microbial amylases to supplement malt in breweries*
	In collaboration with selected breweries in Tanzania microbial amylases will be used to supplement malt enzymes and the quality of the product analyzed.
6.3	*Starch hydrolyzates from cassava, enset, and/or cereal starch test for food application*
	Starch from selected botanical sources will be enzymatically hydrolyzed and different products, produced.
Activity 8: Reporting (technical and financial)	
8.1	*Preparation of technical and financial report*
	Co-PI will prepare annual (and biannual) reports (both financial and technical) and give it to the PI who will compile a full report and submit to the BioInnovate Office.

National University of Rwanda	
Activity 1: Scale up of enzyme production, stabilization, & standardization of enzyme assay conditions	
1.3	*Standardize enzyme assay methods and establish a functioning laboratory*
	To make the laboratory ready to assit industries in Rwanda essential equipment and reagents will be purchased and the method for enzyme analysis standardized.
Activity 5: Evaluation of xylanases and phystases as animal feed additives	
5.1	*Evaluation of xylanases and phytase for poultry & pig feed additives*
	Xylanases and phytases e produced at the pilot plant in Addis Ababa will be tested as animal feed additives using monogastric animals. Growth and feed utilization efficiency of animals feed with enzymes supplement will be compared with control groups and the data analyzed critically.
5.2	*Test phytases as fish feed additives*
	Pytase enzymes will be used to supplement fish feed and the results compared with control groups.
Activity 8: Reporting	
8.1	*Preparation of technical and financial report*
	Co-PI will prepare annual (and biannual) reports (both financial and technical) and give it to the PI who will compile a full report and submit to the BioInnovate Office.

15. Logframe

Title of the Project: **INDUSTRIAL ENZYMES FOR SUSTAINABLE BIO-ECONOMY: Large scale production and application in industry, environment, and agriculture**

Outputs	Outcome	Performance Indicator of Outcome	Data Source	Collection Method	Assumptions s
Objective 1. Scale up production of different industrial enzymes discovered so far using solid state fermentation and submerged fermentation					
Data on large scale industrial enzyme production using solid state and submerged fermentation performance data ready by February 2012	1.1 Companies for the production of industrial enzymes established by the participating institutions alone or in partnership with local and/or international private firms and start to supply the local market by December 2014	The number and amount of enzymes produced in large scale and data on the performance of the optimized production process	• Report on the performance & efficiency of the facility • Video and photographic recordings	• Content analysis • Site visit	• Participating universities willing to put up a commercial enzyme producing company • Local or foreign companies are willing to form partnership
A manual for the disposal of liquid and solid fermentation wastes prepared and made ready for use by December 2013	1.2 Enzyme producing companies utilize fermentation waste for the production of value added products and adopt environmentally safe waste disposal procedures starting from December 2014	Data on the amount of waste transformed for the production of animal feed, biogas, and compost	• Reports from enzyme producers • Site observation	• Content analysis • Visit and interview with enzyme producers	Participating institutions, researchers, or other entrepreneurs establish at least small and medium scale enzyme producing companies soon enough
Objective 2. Scale up downstream processing and optimize methods for enzyme stabilization					
Detailed protocols for enzyme recovery and stabilization prepared and ready for use by December 2013	2.1 Enzyme recovery, stabilization, and formulation carried out locally by local industries and start to supply local markets by April 2014	Data on the level of enzyme recovery, its cost effectiveness, and data on the stability of the enzymes under storage and application conditions	• Report on enzyme recovery • Data on enzyme stability and cost effectiveness of the process	• Content analysis • Interview of end users	• Participating universities are willing to put up a commercial enzyme producing company
Detailed protocol for solvent recovery	2.2 Enzyme producing companies start to recycle	• The amount of solvent recovered • The amount	• Report on solvent recovery process	• Content analysis of the reports • Site visit	•

and waste disposal prepared and ready for use starting from December 2013	solvents and utilize other wastes for production of value added products starting from September 2014	of biogas generated	• Data on the amount of biogas generated from liquid and solid wastes		
Objective 3. Collaborate with industries in the region to evaluate different enzymes under actual application condition					
Report on the performance of all enzymes tested under actual application conditions compiled and made available for users by September 2014	3.1 Leather, textile, pulp and paper, breweries, and starch industries in the Eastern Africa region use locally produced enzymes by April 2015	• Number of industries that participate in evaluation of the performance of the different enzymes • Performance data for each enzyme under actual application condition	• Reports compiled • Performance data for the different enzymes	Analysis of the report and performance data	Commercial scale enzyme production put in place in the region before April 2015

16. BUDGET

The following tables show detailed budget breakdown of the project. Broadly the budget is divided in to two major parts- activities in the different institutions and establishment of the pilot plant. Establishing a pilot plant is extremely expensive and thus only one unit can be purchased in such a project. However, the service will be equally available to all participating institutions.

Budget Title: Industrial enzymes for sustainable bio-economy: large scale production and application in industry, environment
Lead Implementing Institution: Addis Ababa University
Partner Implementing Institutions: UoN, UDSM, NUR, LUND/NIIST-India
Period: 3 year, 2011-2014

APPROVED PROJECT SUMMARY BUDGET

Year 1							
Activity	**Budget Categories**	**AAU**	**UoN**	**UDSM**	**NUR**	**LUND/NIIST-India**	**Total**
A	Equipment and Consumables	276,340	29500	36500	25,100	25,000	**392,440**
B	Travel	2,100	4050	5400	2,700	15,900	**30,150**
C	Field work, training and dissemination	27,660	7300	5000	2,200	-	**42,160**
D	General project expenses	4,200	600	0	-	-	**4,800**
E	Overheads	24,824	4145	2345	3,000	4,090	**38,404**
	Total Year 1	**335,124**	**45,595**	**49,245**	**33,000**	**44,990**	**507,954**
Year 2							
Activity	**Budget Categories**	**AAU**	**UoN**	**UDSM**	**NUR**	**LUND/NIIST-India**	**Total**
A	Equipment and Consumables	247,440	42750	30600	22,900	10,000	353,690
B	Travel	11,400	2700	4450	4,450	27,300	**50,300**
C	Field work, training and dissemination	9,550	10725	8500	1,100	-	**29,875**
D	General project expenses	4,600	0	0	600	-	**5,200**
E	Overheads	21,839	5617.5	2177.5	2,905	3,730	**36,269**
	Total Year 2	**294,829**	**61,793**	**45,728**	**31,955**	**41,030**	**475,334**
Year 3							
Activity	**Budget Categories**	**AAU**	**UoN**	**UDSM**	**NUR**	**LUND/NIIST-India**	**Total**
A	Equipment and Consumables	58,840	21400	6000	4,000	10,000	**100,240**
B	Travel	9,300	4450	3100	5,800	4,000	**26,650**
C	Field work, training and dissemination	15,000	11800	7625	-	-	**34,425**
D	General project expenses	7,200	0	600	-	-	**7,800**
E	Overheads	7,227	3765	866.25	980	1,400	**14,238**
	Total Year 3	**97,567**	**41,415**	**18,191**	**10,780**	**15,400**	**183,353**
	Total Year 1 - Year 3	**727,520**	**148,803**	**113,164**	**75,735**	**101,420**	**1,166,642**

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Annex 1: CV of Amare Gessesse

Department of Biology, Addis Ababa University, P O Box 1176, Addis Ababa, Ethiopia; Tel: +251 911 146855; Fax: +251 111 239471; e- mail: amare.gessesse@gmail.com OR amare@bio.aau.edu.et

PERSONAL DATA

Name:	**Amare Gessesse**
Place of Birth:	Gojam, Ethiopia
Nationality:	Ethiopian
Date of Birth:	February 26, 1960
Sex:	Male
Marital Status:	Married with two children

EDUCATION

- 1999 Ph. D. in Biotechnology
- 1987 M.Sc. in Biology
- 1983 B.Sc. Biology Major, Chemistry Minor

WORK EXPERIENCE

- 2005 – present: Assistant Professor, Biotechnology Program Unit, Addis Ababa University
- 1999 – 2004: Assistant Professor, Department of Biotechnology, Aalborg University, Denmark
- 1987 – 1994: Lecturer, Department of Biology, Addis Ababa University, Ethiopia

GRANTS RECEIVED

- 20007– 2011. Biotechnology and microbial diversity of Ethiopian soda lakes, NUFU, Norway
- 2008 - 2011. Bioenergy for sustainable development, DelPHE/DFID, UK
- 2008 - 2010. Enset agroprocessing, BIOEARN (Sida/SAREC)
- 1993- 1998 Novel industrial enzymes from extremophiles isolated in Ethiopia. Sida/SAREC, Sweden
- 1996 -1998. Industrially useful proteases , IFS, Sweden

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ANNEX 2: CV of Prof. Francis Mulaa

Department of Biochemistry, University of Nairobi P. O. Box 30197, Nairobi, Kenya Phone: +2540204442841, Fax 2540204441186 Email: mulaafj@uonbi.ac.ke // mulaafj@yahoo.com

PERSONAL DATA

Date and Place of Birth:	July 13, 1957 Busia, Kenya
Nationality:	Kenyan
Marital Status:	Single
Languages Spoken:	English, Russian, Swahili, Luhya

EDUCATION

- 1990 Ph. D. in Biochemistry
- 1986 M.Sc. in Biochemistry

WORK EXPERIENCE

- 2006 to date: Associate Professor, Department of Biochemistry, University of Nairobi
- 2002- 2006: Senior Lecturer, Department of Biochemistry, University of Nairobi3
- 1990 – 2002 Lecturer, Department of Biochemistry, University of Nairobi

PUBLICATIONS

1. Julia S. Sabirova, R. Haddouche, N. Van Bogaert, **F. Mulaa,** W. Verstraete, K. N. Timmis, C. Schmidt-Dannert, J. M. Nicaud, and W. Soetaert (2010). The 'LipoYeasts' project: using the oleaginous yeast *Yarrowia lipolytica* in combination with specific bacterial genes for the bioconversion of lipids, fats and oils into high-value products. *Microbial Biotechnology* 1751-7915
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10. Hashim SO, Hatti-Kaul R, Andersson M, **Mulaa FJ** and Mattiasson B (2005). Differential scanning calorimetric studies of a Bacillus halodurans alpha-amylase. *Biochim Biophys Acta*. May 25;1723(1-3):184-91.

11. Johnson K. Kinyua, Edward K. Nguu, **Francis Mulaa** and Joseph M. Ndung'u **(**2005.) Immunization of rabbits with Glossina pallidipes tsetse fly midgut proteins: Effects on the fly and trypanosome transmission. *Vaccine,* 23 (29): 3824-3828.
12. Hashim SO, Hatti-Kaul R, **Mulaa FJ** and Mattiasson B. (2004). Maltohexaose production by a recombinant Bacillus halodurans α-amylase: enhanced yields by in situ product emoval (manuscript).
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Annex 3: CV of Dr. Sylvester Leonard Lyantagaye

Department of Molecular Biology and Biotechnology, College of Natural and Applied Sciences, University of Dar es Salaam, P.O. Box 35179, Dar es Salaam, Tanzania. Phone: (+255) 787537030 / (+255) 712569527, E-mail: lyantagaye@amu.udsm.ac.tz, slyantagaye@gmail.com

EDUCATION

Ph.D. (Biochemistry)	-2005: University of the Western Cape-South Africa
M.Sc. (Appl Microbiol)	-2000: University of Dar es Salaam
B.Sc. (Microbiol and Marine Biol)	-1996: University of Dar es Salaam

EMPLOYMENT HISTORY

- 2007 to date: Lecturer: Department of Molecular Biology and Biotechnology, University of Dar es Salaam
- Feb 2006 – Apr 2007: Postgraduates Teaching, PET project, University of the Western Cape, South Africa.
- May 2005 – Apr 2007: Postdoctoral Fellow, National Bioinformatics Network (NBN), Department of Biotechnology, University of the Western Cape, South Africa.
- Feb 2005 – Apr 2007: Research assistant, Department of Biotechnology, University of the Western Cape, South Africa.

RECENT PUBLICATIONS IN REFEREED JOURNALS

1. Theonest Ndyetabura, **Sylvester Leonard Lyantag**aye and Anthony Manoni Mshandete (2010). Antimicrobial activities of ethyl acetate extracts from edible Tanzanian Coprinus cinereus (Schaeff) S. Gray s.lat. cultivated on grasses supplemented with cow dung manure. *ARPN Journal of Agricultural and Biological Science;* 5 (5)
2. Rose MASALU, Ken M. HOSEA, Mervin MEYER, **Sylvester LYANTAGAYE**, Stonald Kanyanda (2010). Induction of early apoptosis and reactive oxygen species (ROS) production by Tanzanian basidiomycete (Cantharellus miomboensis). *Int J Biol Chem Scis*, 4 (4): 825-833.
3. **Sylvester L**. Lyantagaye and Francis S. Magingo (2010). Stephanostema stenocarpum (Apocynaceae) extract is a potential remedy for bacterial infections in domestic animals. Journal of Medicinal Plants Research, JMPR-10-883 (ACCEPTED)
4. Liberata Nyang'oso Mwita, **Sylvester Leonard Lyantagaye** and Anthony Manoni Mshandete (2010). The effect of the interaction of varying chicken manure supplement levels with three different solid sisal wastes substratse on sporocarp cap length and diameters and dry weights of Coprinus cinereus (Schaeff) S. Gray s.lat. African Journal of Biotechnology. AJB-10-1220, (ACCEPTED)
5. Liberata Nyang'oso Mwita, Anthony Manoni Mshandete and **Sylvester Leonard Lyantagaye** (2010). Improved antimicrobial activity of the Tanzanian edible mushroom Coprinus cinereus (Schaeff) S. Gray s.lat. by chicken manure supplemented solid sisal wastes substrates. Journal of Yeast and Fungal Research. JYFR-10-027 (ACCEPTED)
6. Liberata Nyang'oso Mwita, **Sylvester Leonard Lyantagaye** and Anthony Manoni Mshandete (2010). Cultivation of Tanzanian Coprinus cinereus (Sisal compost mushroom) on three types non-composted sisal wastes supplemented with chicken manure at various rates. International Journal of Biological and Chemical Sciences (UNDER REVIEW)
7. Frankline K. Keter, Stonard Kanyanda, **Sylvester Lyantagaye**, James Darkwa, D. Jasper G. Rees and Mervin Meyer. (2008). In vitro evaluation of dichloro-bis(pyrazole)palladium(II) and dichloro-bis(pyrazole)platinum(II) complexes as anticancer agents. Cancer Chemotherapy and Pharmacology, 63 (1): 139-148.
8. **Lyantagaye SL**, Meyer M, McKenzie J and Rees DJG. (2005). Identification of -methyl D-glucose ether as the active compound from Tulbaghia violacea in the induction of apoptosis. FEBS Journal, 272: 37.
9. **Lyantagaye SL**, Rees DJG. (2003). Screening Tulbaghia violacea extracts for the presence of apoptotic compounds. South African Journal of Botany, 69: 256-257.
10. **Sylvester SL**. Lyantagaye. Chapter-10: Apoptosis Regulation in Mosquito and its Importance to Malaria Infection. In: Raman Chandrasekar. Short Views on Insect Molecular Biology, Vol. (1), 175-190, (2009). International Book Mission Academic Publisher, India.

Annex 4: CV of Laetitia Nyina-wamwiza

PERSONAL DATA

Names: Laetitia Nyina-wamwiza
Date of birth: 17 December 1972
Place of Birth: Idjwi DRC
Nationality: Rwandan
Marital status: Married
Gender: Female

ADDRESS FOR CORRESPONDENCE

Faculty of Agriculture
National University of Rwanda
P.Box. 117 Butare
Mobile: (250) 0788526183
Email: nyinawamwiza@yahoo.fr
lnyinawamwiza@nur.ac.rw
lwamwiza@fundp.ac.be

EDUCATION

- 2007 Ph. D. in Biology
- 2002 M.Sc. Ecology and Technology of Fresh Water
- 1998 B.Sc. Biology

IV. WORK EXPERIENCE

- 2009 – present: Head, Department of Animal Production, NUR
- 2008 -2009: Secretary, Animal Production Department
- 2003 – 2007: PhD student, University of Namur (FUNDP), Belgium
- 1999 – 2001 : Chief technician, Biology laboratory, NUR

V. PUBLICATIONS

1. Laetitia Nyina-wamwiza, Xueliang L Xu, Gersande Blanchard, Patrick Kestemont (2005). Effect of dietary protein, lipid and carbohydrate ration on growth, feed efficiency and body composition of pikeperch *Sander lucioperca* fingerlings. *Aquaculture Research.* ***36****,486-492.*
2. Laetitia Nyina-wamwiza, Bernard Wathelet, Patrick Kestemont (2007). Potential of local agricultural by-products for the rearing of African catfish, *Clarias gariepinus* in Rwanda: effects on growth, feed utilization, and body composition. *Aquaculture Research.* ***38,*** *206-214*
3. Laetitia Nyina-wamwiza, Bernard Wathelet, J. Richir, X. rollin, Patrick Kestemont (2010). Partial or total replacement of fish meal by local agricultural by-products in diets of juvenile African catfish (*Clarias gariepinus)*: growth performance, feed efficiency and digestibilit. *Aquaculture Nutrition* ***16:*** 237 -247**.**