Project Description

1. Identification and significance of the innovation

This project proposes a two-stage culture process of heterotrophic microalgae with a high cell density of more than 100 g/L to produce omega-3 fatty acids from crude glycerol, a byproduct from the biodiesel industry. The project is innovative in that a unique two-stage high-density marine algae culture process has been developed and used in the DHA-enriched algae biomass production and the feedstock for the production utilizes crude biodiesel; allowing for a much needed market for the low to negative value biodiesel byproduct. This proposal is being submitted under the Biotechnology topic area and sub-topic area ‘I’ entitled ‘Aquaculture and Aquatic Biotechnology’ because development of a process for marine algae utilization to produce a high value product such as omega-3 fatty acids fits well within the sub-topic goal of “development of products beneficial to human health from marine and other aquatic environments with minimal impact to such environments” and this process is an “application of biotechnology to aquaculture” since the produced algae biomass will be used as an aquaculture fish feed supplement.

In previous studies culturing marine algae *Schizochytrium* performed at Washington State University (WSU) (project subcontractor), it was found that the growth of this algae can be divided to two stages: (1) a cell number increasing stage: cell proliferation and rapid cell number increase with little increase in the size and weight of each cell and (2) a cell size increasing stage: cells stopped proliferation and enlarged due to fatty acids accumulation. Notably, the optimal culture conditions for the two stages are different. Based on this discovery, separate optimizing culture conditions of the two stages were developed, and by using a “shifting” strategy between these phases, significant increases in cell culture density were produced. More than 100 g/L algae dry biomass was obtained in the subcontractor’s lab scale studies; showing great commercial potential of this process.

This discovery of a two-stage growth of *Schizochytrium* could significantly improve the general understanding to the relationship between biomass production and lipid accumulation in lipid-producing microorganisms, especially microalgae. Previously, it was considered that lipid accumulation in the cell requires the exhaustion of some essential nutrients in the culture medium, and limiting them is an effective way for inducing lipid accumulation (Granger, *et al.*, 1993). However, this procedure also results in decreases in total productivity due to the overall effects on growth by the nutrient limitation. The U.S. Department of Energy’s Aquatic Species Program (ASP) which conducted a series of studies on biodiesel production from algae made a conclusion that “studies of lipid accumulation suggest that an understanding of the kinetics of the process could be critical and could allow the identification of a stage where biomass productivity and lipid levels are optimal for maximal lipid accumulation” (National Renewable Energy Laboratory, 1998). With the discovery of two-stage growth, however, cell proliferation and lipid accumulation could be optimized separately with a two-stage culture, rather than one stage in which lipid accumulation conflicts with cell proliferation. This two-stage culture process could be used not only in the omega-3 fatty acids production process, but probably more widely,
in all single cell oil (SCO) producing processes, including biodiesel production from algae, which is the largest potential SCO application.

In regard to the innovative use of crude glycerol, successful application of the proposed process could have a significant impact on the biodiesel industry as it helps to solve the problem of waste glycerol treatment in the biodiesel industry. For a current biodiesel production of 150 million gallons/year, 150 million pounds of crude glycerin could be produced annually (Pachauri and He, 2006). This waste glycerol will be a threat to the environment if it cannot be properly treated and additionally will be an added burden to burgeoning biodiesel industries because of its negative value—present open market prices for received crude glycerol are at 5 cents/pound and conceivably could drop even further as production ramps up (personal communication with Seattle Biodiesel). Converting this waste glycerol into value-added bioproducts is definitely a better choice for treatment of this glycerol. Since utilization of the biodiesel waste leads to increased economic viability of the biodiesel industry as well as reduction in the potential impact to the environment for the disposal of this waste stream, the project results become one related not to just biotechnology improvement but industry, security, and energy enhancement.

The DHA heterotrophic algae producer will benefit from this project, since using the crude glycerol as the carbon source will significantly reduce the cost of the algae culture process. Currently, although the heterotrophic algae culture process is taking on a more and more important role in omega-3 production, fish oil is still a substantial threat to this developing technology because of its low production costs (Martek Annual Report, 2004). Additionally, several companies have developed microencapsulated fish oil products that claim to have resolved much of the odor, stability and taste issues associated with fish oil while simultaneously removing the need for costly purification. As a result, decreasing the production cost of the algae culture process remains an indispensable work for the DHA heterotrophic algae producer, and this project will provide a practical way by using the crude glycerol as an inexpensive carbon source.

This project will also improve the development of an “organic fish” industry, in that it will provide an inexpensive source of omega-3 that does not originate from fish meal. In fact, Omega-3, although widely used in the pharmaceutical, nutraceutical, and food industries, owes its biggest market share to aquaculture fish feed. At present, fish oil production amounts to about 1 million tons annually, of which 70~80 % is utilized for the production of fish feed for farmed fish (Guzman, 2006).

Currently, farm-raised fish obtain omega-3 only from fish meal. As the aquaculture feed demand increases and ocean fishery resources decline, using fish meal to support aquaculture growth becomes non-sustainable. In addition, the development of an organic fish movement requires an omega-3 source that does not originated from fish meal. Therefore, feeding an organic diet supplemented with enriched omega-3 algae becomes almost the only future option for the aquaculture industry.

2. Background and phase I technical objectives

The utilization of crude glycerol from the biodiesel industry

During the typical biodiesel production process oils and fats from oilseeds and the oleo-industry
are mixed with methyl alcohol and alkaline catalysts to produce biodiesel, with glycerol as a primary waste product. The mass conversion of this process is approximately described by 100 units oil/fat plus 10 units alcohol to produce 100 units biodiesel and 10 units of glycerol. The results are that for every 7.35 pounds of oil/fat converted, 1 gallon of biodiesel and 1 pound of crude glycerol (80-70% pure) are produced (Pachauri and He, 2006). With further development of the US biodiesel industry, it is anticipated that medical and industrial-grade glycerol prices will drop significantly because 1 billion pounds of biodiesel glycerol could be introduced into market by 2007 whereas currently the total commercial market for glycerol is only half of that projected production. Clearly, then, the biodiesel industry will be in need of alternative uses/markets for this waste glycerol. Add in the fact that this byproduct glycerol is in a crude form containing many impurities and a situation exists where there is not just a surplus of glycerol, but a surplus of crude glycerol that is expensive to be purified to higher quality. As stated earlier, this troublesome scenario is in fact playing out with crude glycerol receiving open-market prices of only $0.05/lb.

**Omega-3 polyunsaturated fatty acids and DHA**

Omega 3 polyunsaturated fatty acids (ω-3 PUFAs) are a group of fatty acids containing two or more double bonds, of which the last double bond is located at the third carbon atom from the methyl terminal. For a long time, the beneficial effects of ω-3 PUFAs have been recognized by epidemiological surveys that revealed Eskimos—who consume large amounts of deep-sea fish—rarely suffered from heart diseases. Docosahexaenoic acid (DHA, 22:6) is one of the more important ω-3 PUFAs, with a 22 carbon chain and 6 double bonds and is known to have particular beneficial effects in fetal and infant brain and ocular development. The inclusion of supplementary DHA in infant formulas is strongly recommended by the World Health Organization (WHO) (FAO/WHO Expert Committee, 1994). Also, research continues to demonstrate the need for DHA beyond infancy, with studies suggesting a positive correlation between DHA consumption and the reduced risk of age-related neurological disorders, such as Alzheimer’s and dementia. As a result, DHA is not only used as additives in infant formulas, but also in adult dietary food and beverage supplements. Example foods are cheeses, yogurts, spreads and dressings, and breakfast cereals. Notably, these markets may have much greater growth potential than infant formulae; thereby substantially elevating the entire DHA market potential.

The conventional source of ω-3 PUFAs is mostly from fish oil. Cod, salmon, sardine, mackerel, menhaden, anchovy, tuna, and seal are generally used for fish oil production. The quality of fish oil depends on fish species, seasons and geographical locations of catching sites. As marine fish oil is a complex mixture of fatty acids with varying chain lengths and unsaturation degrees, DHA needs to be refined from fish oil for use in nutraceutical/pharmaceutical applications. The purification of DHA from low-grade fish oil is difficult and costly (Belarbi et al., 2000) although new micro-encapsulation procedures are now in development. In addition, marine fish stocks are subjected to seasonal and climatic variations, and may not meet the requirement for providing a steady supply for the increasing demands of DHA.

**DHA enriched biomass as feed additives in aquaculture**

Currently, fish oil is a main ingredient in finfish and marine shrimp feeds, mainly because they offer a range of fatty acid classes, including omega 3 which contribute to the energy, growth, and
reproductive demands of the fish. However, the level of PUFA in fish oil varies depending on species, extraction procedure and storage conditions. In fact, standard available fish oils do not offer sufficient levels of DHA ratios to completely satisfy the nutritional demands for reproduction and larval growth (Harel et al., 1994). Consequently, marine oils where the DHA levels are particularly high due to its origin from specific fish tissues, or through special extraction procedures, have been recommended in broodstock diets and larval rearing enrichment preparations, but the availability of these high DHA-containing oils is limited and often prohibitively expensive to produce and the idea of catching fish to feed fish is not especially environmentally friendly. So, the aquaculture industry is now actively investigating alternative nutrient sources, with land-based vegetable and unicellular organisms such as microalgae, yeast, molds, bacteria, and fungi being taken into consideration (Harel et al., 2002).

A major advantage in the use of unicellular organisms is that they can be easily produced in industrial quantities under controlled and environmentally safe conditions and studies have shown that heterotrophic algal and fungal supplemented diets are highly effective in delivering essential fatty acids either through larval live food enrichment or directly through the fish diet (Harel et al., 2002). Unlike fish oil, which contains various omega-3 and other polyunsaturated fatty acids (PUFA) depending on species, extraction procedure and storage conditions, the dried heterotrophic algae and yeast usually have a very stable rate of PUFA. Furthermore, DHA can be produced by different groups of algae strains separately, and combinations of these alga strains can offer a broader range of fatty acids to meet more effectively the species-specific dietary requirements, which is not realizable with fish oil (Harel et al., 2002).

Another potential use of heterotrophic algal preparations is that they could be very useful in hatcheries of marine finfish, shrimp and oysters to partially substitute or supplement a live algae diet of the larvae, which is very costly with the photosynthetic algae. The partial replacement of up to 40% living algal supplements using spray-dried heterotrophically-grown algal biomass have been reported for a number of marine organisms, such as mussel, juvenile bivalve mollusks, and juvenile clams (Boieng, 1997).

**DHA production from algal heterotrophic culture**

It is known that fish, like humans, are not capable of synthesizing PUFA *de novo*. Much of their PUFA is derived from the primary producer in the oceanic environment: the microalgae or algae-like microorganisms. There are a large number of microalgae containing DHA in nature, but only a few species have demonstrated production potentials on an industrial scale. This is mainly due to the low specific growth rates and low cell density of the algae, as they can only grow, in many cases, in photoautotrophic conditions.

Intensive research into the production capabilities of these microalgae led researchers and commercial industries to focus on and develop heterotrophic algal production processes for DHA. As of late, the two algae used commercially or showing the greatest commercial promise are the heterotrophic dinoflagellate *C. cohnii* and strains from the *traustochytrid* marine protists. Developments of commercial processes for production of DHA with these two algae has benefited from the fact that they can accumulate high oil contents in their biomass (10–50%, w/w) and produce a high percentage of total lipids as DHA (30–70%). High biomass densities (up to 109 g/L) and DHA concentrations of 20 g/L have been achieved in carbon fed batch
cultures of marine species, *C. cohnii*, although prolonged culture times (400 h) were required. These studies have demonstrated that DHA productivities of 1–1.5 g/(L day) are achievable with this strain (Ward and Singh, 2005).

The best microbial sources of DHA, though, are from *Thraustochytrids*, specifically the genus *Thraustochytrium* and *Schizochytrium*. *Thraustochytrium* and *Schizochytrium* are unicellular algal or algal-like protists, members of the order *Thraustochytriales*; family *Thraustochytriaceae*; genus *Thraustochytrium* or *Schizochytrium*. *Schizochytrium* replicates by both successive bipartition and by release of zoospores from sporangia, whereas *Thraustochytrium* strains only replicate by formation of sporangia/zoospores. Studies with *thraustochytrids* have established these marine protists as preeminent industrial strains for production of DHA. Initial research, at relatively, low cell densities, (5–20 g/L) established the capacities of *Thraustochytrium* species to accumulate greater than 50% of their lipids as DHA and to produce >1 g DHA/L of culture, with productivities of about 0.2 g/(L day) (Ward, 2005). Lately, *Schizochytrium* species with higher growth rates have been isolated. Under glucose and nitrogen-fed batch conditions, with incorporation of sodium sulfate as a main sodium source and with control of glucose concentration, pH and oxygen, selected strains have been shown to grow to high biomass densities (200 g/L) in short fermentation cycles (90–100 h), accumulating 40–45 g/L DHA and DHA productivities of >10 g/(L day). These excellent performances have made *Schizochytrium* the major producer in the DHA industry (Bailey *et al.*, 2003).

**Previous WSU subcontractor lab scale work results**

In WSU’s previous work, the culture condition of *Schizochytrium limacinum* SR 21 with crude glycerol as the feedstock was optimized and a fed batch culture process was developed and a high cell density culture (>100 g/L) has been realized at a lab-scale.

1. **Oxygen supply protocol optimization**
   The oxygen uptake rate in the different growth stage of this alga was investigated with both continuous culture and batch culture in a fermentor with dissolved oxygen (DO) control. It was found that high oxygen consumption is required in the cell propagating stage, when cell number increases but little increase occurs in the size of the cell. A second stage of cell growth occurs and it is at this stage with low oxygen uptake that the fatty acid accumulates. An optimized oxygen protocol has been developed to produce more cells (control at 50% DO) and then provide a best condition (<5% DO) for fatty acid accumulation. With this protocol, 37.9 g/L dry algae biomass was produced.

2. **Fed-batch culture protocol development**
   A fed-batch culture protocol was developed, since the increased cell number needs more nutrients to accumulate fatty acids and reach higher cell density. In this fed batch culture, 25 g/L glycerol and 25% of the initial nitrogen and salts were supplemented to the culture daily, and the final cell density was increased to 55.6 g/L. With more seed cells as initial cells, a bioreactor culture with fed batch protocol eventually attained a cell density of 102 g dry algae biomass per liter broth.

**Phase I Technical Objectives**

High cell density culture is obligatory for a production process to be cost effective. Disregarding
the work by WSU, there have only been two reports on algae being cultured in a cell density of more than 100 g/L (Ward and Singh, 2005). WSU’s results and process therefore are a very important breakthrough. It is notable that the 100 g/L cell density in WSU’s study was obtained with a not fully optimized culture condition. In fact, besides dissolved oxygen, which was optimized in that study, culture conditions such as temperature, nitrogen source and its concentration also have significant effects to both the cell proliferation and fatty acid accumulation. In fact, these works are undergoing in WSU’s lab and promise for attaining even higher productivities has been shown in these studies. These works will be accomplished before this phase I study. After that, to further refine and prove this process as well as commercialize this process, work needs to be done on: (1) scale-up, (2) decreasing the production cost, and (3) assessing the overall economic viability. To that end, the technical objectives of the phase I work will be:

(1) Culture medium component refinement  
The components in the culture medium in the lab scale work included a carbon source (crude glycerol), nitrogen sources, sodium chloride, and various mineral salts from artificial seawater. It is notable that not all of these mineral salts are necessary for the *Schizochytrium*’s growth. Getting rid of the dispensable mineral salts and reducing the amount of indispensable ones is important to reduce the cost of feedstock in this process. Also, the chemicals used in the lab scale and industrial scale are different because of the different content of impurities. Testing the effectiveness of the more inexpensive bulk chemicals used in the industrial scale is necessary. Sodium chloride is not preferred in the industrial process in that the chloride ion could cause corrosion of the stainless steel fermentor and agitator. Thus reducing the amount of chloride ion by substituting it with sodium sulfate needs to be tested and evaluated.

(2) Scale up of the algae culture process  
The dissolved oxygen (DO) concentration will become the limiting nutrient in processes of high oxygen demand (fast growing microorganisms, high biomass) or when the rheological properties of the broth offer a high resistance to the mass transfer. The cell density in the later stage of this algae culture process is more than 100 g/L and although less oxygen is consumed for the fatty acid accumulating step, such high cell density, on the whole, will require a large amount of oxygen. Also, this high cell density probably will change the rheological properties of the broth, which may offer a high resistance to mass transfer and decrease the oxygen transfer rate.

The dissolved oxygen (DO) in the broth is limited by both its consumption rate on cells (OUR) and its transfer rate (OTR). The mass balance of oxygen can be described as:

\[ \frac{dC_o}{dt} = OTR - OUR = K_{L*a} \cdot (C* - C) - (Q_{o2} \cdot C_x) \]

The OTR could be affected by geometry and characteristics of the vessels, liquid properties (such as viscosity), the dissipated energy in the fluid, biocatalyst properties, concentration, and morphology of microorganisms. In a specific fermentor and medium, the OTR value mainly depends on the air flow rate and the agitation speed. In the above equation, \( K_{L*a} \) represents the ability for oxygen transfer in a specific fermentation system and often serves to compare the efficiency of bioreactors and mixing devices as well as being an important scale-up factor. In fact, fixing \( K_{L*a} \) values is a commonly used criterion for scale-up of aerobic fermentations.
(3) Analysis of the economical viability

To meet the market requirement, the quality of produced algae biomass needs to be analyzed, in term of nutrient ingredients, fatty acids profile, vitamin profile, etc, and the value of the product need to be assessed. Based on the calculation and comparison of production cost and produced algae biomass value, an economical viability of this process needs to be evaluated.

3. Phase I research plan

It has been proven that this algae culture process with crude glycerol as the raw material is feasible in the lab scale. Thus, the overall objective for this phase I research is to scale up this process, and assess the economical viability of this process. The work on refining the medium components will be conducted by the subcontractor, Washington State University, and the work on scaling up of this process and assessing its economic viability will be conducted by AEB Engineering, LLC.

Objective 1: Refining the medium components

A. Reducing the medium components:

Besides carbon source and nitrogen sources, a series of mineral salts from artificial seawater are involved in the culture medium. Some of these mineral salts are useful while probably some are not. A Plakett-Burman design will be conducted to investigate every element’s effect to this culture. In this experiment, the cultures with and without certain elements will be compared, and statistical analysis will be made to determine whether each factor is significant. The non-significant factors in this experiment will be removed from the culture medium. For the significant factors, further studies will be conducted to decrease their contents to as low as possible.

B. Testing the feedstock from industry:

For the carbon source, different crude glycerol obtained from different seed oil feedstocks, such as mustard, rapeseed, canola, crambe, soybean, and waste cooking oils will be used in the algae culture processes and be compared (samples will be obtained from industry partners at Seattle Biodiesel and the University of Idaho). The effect of different crude glycerol source to the algae’s growth will be investigated. There are different impurities involved in the crude glycerol from different sources. The effect of these impurities will be determined, then.

Besides the carbon source, the nitrogen source and necessary mineral salts from the industrial scale will be compared with that used in the lab scale. If there is a difference, the effect will be further investigated, until the proper chemicals used in the industry scale are selected.

C. Reducing the sodium chloride concentration in the culture medium

Reduction and/or removal of sodium chloride from the industrial-scale culture medium will be accomplished by investigating the effect of sodium chloride to the algae’s growth, from the range of 18 g/L to 0 g/L, and then optimizing the concentration based upon the experimental results. After optimization, further reduction in the sodium chloride amount will be accomplished by studying the effect of sodium sulfate substitution (100% to 0%). With this optimization, the concentration of sodium chloride will be reduced to as low as possible, as long as there is no obvious negative effect to the algae’s growth.
**Objective 2: Scaling-up the laboratory scale DHA production process to pilot scale**

Scale-up using the new optimized media obtained in objective 1 will be accomplished using 1 L, 25L, and 125L fermentors within the Bioprocessing Service Center Laboratory at WSU. For the oxygen transfer issue, the OUR, OTR, and \( K_{La} \) values in both the seed cell producing stage and fatty acid accumulating stage will be investigated at various aeration and agitation rates in a 1 liter lab scale fermentor (0.1–1.0 vvm and 200–500 rpm, respectively) as well as in a 25 L fermentor (0.1 –1.0 vvm and 200–500 rpm, respectively). The \( K_{La} \) values of both scales will be compared to find out a control condition that gives the same \( K_{La} \) for scale-up study. The effect of \( K_{La} \) to the algae’s growth and fatty acid accumulation in the 1L, 25 L, and 125L fermentors will be recorded and compared. Problems such as oxygen concentration gradients in the fermentor in this scaling up process will be recorded and investigated if it is encountered.

**Objective 3: Economic viability assessment**

The produced algae in the pilot study will be washed and dried by a spray dryer. Then, the component in this algae dry biomass will be investigated with analysis of protein, carbohydrates, fatty acid profile, vitamins, sterols, and amino acid profile. The price of the produced algae biomass will be evaluated based on their quality with comparison to present market prices.

The cost of raw materials, energy, labors, as well as the process productivities etc. in the pilot study will be recorded and studied. A techno-economic assessment of the feasibility of DHA enriched algae biomass production will be conducted using Matlab Simulink software using the laboratory and scale-up results and standard chemical engineering plant design and costing concepts as summarized by Peters and Timmerhaus (1991).

**Phase I Performance Schedule**

The project objectives will be accomplished in 6 months following the timeline below:

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<th>Objective</th>
<th>Content</th>
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<td>Refining the medium components</td>
<td>2 months</td>
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<tr>
<td>2.</td>
<td>Scale-up and pilot study</td>
<td>3 months</td>
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<tr>
<td>3.</td>
<td>Economic viability assessment</td>
<td>1 months</td>
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**4. Company information**

AEB Engineering, LLC is the company submitting this proposal. AEB Engineering was formed on November 4 of 2005 in response to a perceived need to carry forth concepts developed at the Department of Biological Systems Engineering at Washington State University (WSU). Founding partners are Mr. Craig Frear who will be manager on this grant if awarded and Dr. Shulin Chen who will supply chief technical expertise as well as supervision of subcontract work to the WSU laboratory while serving in his role as professor at WSU. The mission of the company is to facilitate commercialization of industrial biotechnology solutions to agricultural and environmental concerns, particularly through the research and development of novel bioproduct production systems. Specific areas of expertise involve synergetic mixed microbial fermentation, enhanced fermentative process control, enhanced biocatalysis through
pelletization, mathematical modeling for production and design enhancement, and innovative reactor design.

AEB Engineering as a relatively recent start-up company presently has no employees other than the founding members of the LLC and specifically Mr. Craig Frear, the acting manager. The AEB Engineering business plan is to use the manager’s time to write and potentially obtain SBIR type research funding so that laboratory findings can be scaled up and further refined for eventual sub-licensing to interested industries and businesses. While working on the SBIR type research, the manager will also devote considerable time in attaining intellectual property rights and patent protection for selected technologies developed within WSU as well as developing industrial contacts for eventual sub-licensing and marketing of the technologies. Attainment of this proposed SBIR grant would have considerable influence in furthering this business plan, specifically allowing for funding of an initial employee to perform research and allowing for development of a technology ready for sub-licensing and sale.

Agreements already exist between WSU, the WSU Research Foundation, and AEB Engineering, LLC for AEB Engineering to be the sole licensee of the intellectual property described within the proposal for which the WSU Research Foundation has ownership. As part of that agreement, the WSU Research Foundation will receive a nominal contractual fee as well as part ownership in the company and agreed upon royalty transfers from any assets realized from the sale and/or sub-licensing of the technology/products. Patent protection and sub-licensing is agreed to be at the discretion of AEB Engineering, LLC with guided input from the WSU Research Foundation. As per University regulations regarding conflict of interest, the appropriate forms have already been filed with the WSU Office of Grants and Research Development regarding Dr. Chen and Mr. Frear’s involvement as both University employees and business partners and their potential use of university service center laboratories. Specifically, fiduciary oversight of the sub-contract tasks within the WSU laboratory will fall, as per negotiated agreement, to the WSU Departmental Chair in charge of the WSU laboratory.

In regard to technical expertise, Dr. Chen is a full professor of biological systems engineering and a registered PE within Washington State with over 20 years of active research experience in this field and specifically within biomass utilization and fermentative conversion. Mr. Frear is a fourth year PhD graduate student in Biological Systems Engineering with five years of past research associate experience in researching and managing multiple research grants and projects and being an office and departmental manager with fiduciary oversight.

5. Commercial potential
There is a great market potential for the products resulting from the project. The omega-3 enhanced feed can be used virtually for all types of animal operations. Studies have shown that dairy cattle will gladly tolerate up to a 2% algae supplement to their diet with the desired omega-3 fatty acids capable of being transported across the rumen into the milk and meat supply (Chilliard, Y., 2001). With the large dairy and cattle industry, there is more than enough of a market to meet the algae-omega-3 production capabilities. The availability of Omega-3 in animal product will give the product value added competitive advantages at the market place that will help to improve the farm income.
Of particular note, though, is the possibility of inclusion of algae as a supplement to the feeds of other animals, especially farm-raised fish. In 2001, 819 million pounds of aquaculture fish were grown in the US yielding over $935 million in sales. These numbers are growing dramatically at an annual rate above 20%, making it according to the USDA Agriculture Statistics Service, “the fastest growing sector in US agriculture.” The growth is a result of worldwide declines in fish catch, concerns with toxins within the meat of the catch, and a need to supply a rapidly growing world with a ready source of protein and healthy fats. The growth of the global aquaculture industry is also driving increased demand for both fish oil and meal, and the feed manufacturers are now taking 70% to 80% of the total 1 million tons of fish oil globally produced.

As far as the competitive advantages concerned, the omega 3 algae produced in this process could be instrumental in meeting the unique needs of the burgeoning aquaculture industry, rather than the currently used fish oil. The marketability of their aquaculture product relies on an ability to emphasize the healthy aspects of the meat. Fish, although prized for their high omega-3 content, only obtain these fatty acids through a diet of algae, the primary producers of omega-3. As the aquaculture feed demand increases and ocean fishery resources decline, using fish meal to support aquaculture growth becomes non-sustainable. In addition, the development of an organic fish movement requires an omega-3 source that does not originate from fish meal. Therefore, feeding an organic diet supplemented with enriched omega-3 algae becomes almost the only option for the future aquaculture industry.

In regard to providing a competitive DHA-enriched algal cultivation process to companies already active within this field, as noted earlier, to be competitive in the existing market, algal cultivation processes must further reduce their process costs to stay competitive, and it is believed that this technology proposed here has a strong potential for reducing those costs. The main reasons for this belief and competitive advantage are: (1) the development of a high cell density production process that already at the lab-scale can reach productivities of greater than 100 g/l and (2) the use of a low to negative cost crude glycerol feedstock during the process. It is believed that both of these selling points will be of considerable interest to industrial players within this field and that scale-up and/or incorporation with other industrial devices such as genetically-engineered organisms, etc. will allow for even higher productivities at reduced process costs. It was reported that the production cost of algae dry biomass has been reduced to less than $5/kg, and even as low as $2/kg (Glaude and Maxey, 1994). It is well known that the conversion rate of carbon source to algal biomass is usually less than 50%. Thus, to produce 1 kg algae biomass, at least 2 kg of carbon source need to be consumed. If taking the glucose as the carbon source, which is priced at $0.06/lb, the cost of 2 kg glucose is $0.26, which accounts for 13% of the total production cost of $2. Thus, using the cheap or even negative value crude glycerol as the carbon source will significantly decrease the production cost while also providing an effect way to dispose of the large volumes of crude glycerol produced during the biodiesel process.

The potential customer and commercial plan if the phase I research and development warrants further work and proof of concept is to have AEB Engineering, which will hold patent and intellectual property licensing rights, sub-license all or parts of the developed technology to existing omega-3 algae producers, be they nutraceutical or aquaculture feed suppliers in the case of extracted or non-extracted forms, respectively. AEB Engineering’s business plan is to not be a
producer and marketer of products but to be a developer of innovative processes and technologies and to sub-license them out to companies and industries which are well situated to produce and market the products that derive from the innovative processes and technologies.

AEB Engineering has been formed with efficient transfer capabilities of intellectual property in mind. Initially, much of the processes being developed will have derived from research completed at WSU since the founding partners are the researchers at WSU. Since the research was developed at WSU the intellectual property rights transfer to WSU and its Research Foundation, but the WSU Research Foundation is a partner in the company thus allowing for pre-arranged agreements for equitable transfer of the rights to the company. Now holding, the intellectual property licensing rights, AEB Engineering is then free to apply for patent protection and sub-license once further proof of concept has been attained through such grants as the one applied for here.

6. Consultants and Subcontracts

Consultant
There are no consultants on this project.

Subcontract
AEB Engineering will be subcontracting a portion of the work to the Washington State University Department of Biological Systems Engineering. The subcontract will be supervised by Dr. Shulin Chen. He is the coordinator for the agri-environmental and bioproducts engineering (AEBE) research group which has significant expertise in the field of algal fermentation. Sub-contract work within the WSU service center laboratory housed within the department will concentrate on Objective 1 and the utilization of available fermentors as required in Objective 2. AEB Engineering, for its part will be focus on development and completion of Objectives 2 and 3 as well as compiling all of the information and writing the required final report.

7. Equivalent of Overlapping Proposals to Other Federal Agencies

NONE