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Bioenergy to save the world - Novel energy plants for growth on abandoned land. An Opinion paper*

Peter Schröder[§], Rolf Herzig, Bojin Bojinov, Ann Ruttens, Erika Nehnevajova, Stamatis Stamatiadis, Abdul Memon, Andon Vassilev, Mario Caviezel and Jaco Vangronsveld

Abstract

Background and Aim - Following to the 2006 climate summit, the European Union formally set the goal of limiting global warming to 2 degrees Celsius. But even today, climate change is already affecting people and ecosystems. Examples are melting glaciers and polar ice, reports about thawing permafrost areas, dying coral reefs, rising sea levels, changing ecosystems and fatal heat periods. The Stern review (<http://www.sternreview.org.uk>) concisely reviews global climate change and addresses the issue of increasing atmospheric CO₂ concentrations caused by human activities. Within the last 150 years, CO₂ levels rose from 280 ppm to currently over 400 ppm. The review notes that if we continue on our present course, CO₂ equivalent levels could approach 600 ppm by 2035. However, if CO₂ levels were not stabilized at the 450-550 ppm level, the consequences could be quite severe. Hence, if we do not act now, the opportunity to stabilise at even 550 ppm is likely to slip away.

Results - Long-term stabilisation will require that CO₂ emissions ultimately be reduced to more than 80% below current levels. This will require major changes in how we operate. CO₂ concentrations will not decrease unless stronger agreements than the Kyoto Protocol materialises. Reducing greenhouse gases from burning fossil fuels seems to be the most promising approach to counterbalance the dramatic climate changes we would face in the near future. It is clear since the Kyoto protocol that the availability of fossil carbon resources will not match our future requirements. Furthermore, the distribution of fossil carbon sources around the globe makes them an even less reliable source in the future. Together with the economic fact that energy and raw material prices have drastically increased over the last decade they necessitate the development and the establishment of alternative concepts.

Discussion – We propose to use crop and non crop species to produce mutants that will be screened for high biomass production and good survival on marginal soils. These plants, when grown in adequate crop rotation, will provide local farming communities with biomass for the fermentation in decentralized biogas reactors, and the resulting nitrogen rich manure can be distributed on the fields to improve the soil. Such an approach will open new economic perspectives to small farmers, and provide a clever way to self supporting and sustainable rural development.

Conclusions – Biotechnology is available to apply fast breeding to promising energy plant species. It is important that our valuable arable land is preserved for agriculture. The opportunity to switch from low income agriculture to biogas production may convince small farmers to maintain in their business and by that preserve the identity of agricultural communities. Overall, biogas is a promising alternative for the future, because its resource base is widely available, and single farms or local cooperatives might start biogas plant operation.

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Introduction

The World Energy Outlook 2006 (www.iea.org) specifies that the world will have to face two energy-related threats: the first of not having adequate and secure supplies of energy at affordable prices and the second of environmental harm caused by consumption too much of it. Hence the G8-leaders postulated the need to decrease fossil-energy demand in favour of geographic fuel-supply diversity. Mitigating climate-destabilising emissions is more urgent than ever. They called on the IEA to “advise on alternative energy scenarios and strategies aimed at a clean, clever and competitive energy future” (IEA 2006). Although the outlook paints a dark picture of the development until 2030, it makes a clear statement that bio-fuels and renewables could make up to 12 % of the global energy budget if the right measures will be taken. Biogas produced from animal manure, wastes and fresh biomass ranks amongst the most sustainable energy forms, especially because of its potential production in small, decentralized units that require no big transport infrastructure. But the report does also state clearly, that increasing food demand, which competes with biofuels for existing arable and pasture land (see also Johansson and Azar, 2007), will constrain the potential for biofuels production using current technology. Hence it is clear that sustainable and efficient biomass production must not proceed on valuable arable land but on so far neglected and set aside plots. The latter means that solutions have to be proposed for soils that have been abandoned, e.g. because they may be degraded, polluted or non-exploited, relatively poor soils.

Similarly, the EREC (European Renewable Energy Council) declares that adopting the targets for the share of bioenergy will be the basis for the continuation of a sustainable EU energy policy, as well as a precondition for establishing a sustainable, competitive and secure energy mix for the future (EREC 2006).

In 2003, the European Commission initiated the European Technology Platform "Plants for the Future" as a stakeholder forum on plant genomics and biotechnology. This platform is supported by the European Commission and the major public and private stakeholders. It is coordinated by EPSO (www.epsoweb.org/catalog/tp/). Several high ranking European plant physiologists sketched their opinion about future developments in food and energy crops. It becomes clear that we do presently not fully exploit the potential in plants, and that we can be able to provide the world with sufficient nutrients and energy when we boost biotechnology of plants (<http://www.europabio.org/relatedinfo/Plantgenbrochure.pdf>). However, such a new generation of genetically modified plants will bring a new wave of concern about the safety of the global food, and feed supply. Providing the world with bioenergy using more conventional

ways of classical breeding is another option which has been postulated even earlier. Ambitious papers on the production of biogas from green and animal manure have been published as early as in the 1980ies (Drake 1983, Long 1984).

European border countries and countries of the Mediterranean will encounter particular problems when trying to adopt bio-energy plants to fight climate change, because their production systems are concentrated to areas of high fertility and water availability. Hence, substituting crops for energy plants will lead to shortage in food production and soil resources. The most abundant source of plant-derived energy is where most of the fixed carbon is deposited- the cell wall. Additionally new technologies can be developed to convert cell wall material in fuels. Progress is likely to result from a combination of plant breeding, plant and microbial metabolic engineering, and chemical engineering. On the plant side, for example, a promising approach is the development of ways to modify cell wall composition to create a “feedstock” that is more readily converted into fuels.

Upcoming concepts for energy plants

There are numerous approaches to reduce CO₂ emissions from fossil fuels, but the use of bio-based strategies to fulfil our energy demands is most promising. Recent literature has identified several crops as possible energy plants, amongst them maize, wheat, potatoes, rapeseed and others, that can be produced on European arable land to substitute fossil fuels and make the European energy market more independent. Technologies for producing bio energy from plants, their by-products and from manure are well established, but the crop plants under consideration have been specifically selected for food and fodder production and hence produce high value protein, oil or carbohydrate. As long as our concepts for energy plants are based on high yield cereals and other quality livestock feed the cost of the energy produced will remain far too high to become competitive. If it wants to compete on a global scale, Europe needs novel plants to produce cheap biomass and green manure but leave our food base untouched.

Research on the production of novel energy plants requires (a) identification of plant species or mutants producing biomass in the optimum composition for the bioenergy market, e.g. biogas, (b) testing the agronomic capabilities of the chosen bioenergy plants, (c) studying how to produce bioenergy from the chosen species to demonstrate their potential, and (d) testing their performance on marginal or abandoned land, in order to present a sustainable and financial attractive alternative to the existing energy plants to the stake-holders. Only if these

conditions are met, the initially identified bioenergy plants will succeed, biotechnology will be strengthened, and we will be successful in contributing to solve the world's energy problem.

Bioenergy options - Current attempts to replace mineral oil, coal and gas by fuels derived from agricultural plant production seem most efficient. Bio-ethanol production, Biodiesel (i.e. rapeseed oil methyl ester) and Biogas (i.e. methane and aliphatic gases) from plant sources are in the focus of interest. Production of energy crops is an already ongoing process, and technologies for bio-fuel production from crops are established in richer countries. Biogas production may be an alternative for poorer regions because the fermentation process can be performed decentralised on every farm and will deliver heat, gas to be stored and electrical energy. Hence it will be of incredible potential for rural areas of low standards and with lacking infrastructures.

As reviewed by Markard et al. (2002), Biogas from agriculture has many ecological advantages. Being renewable, it may substitute fossil energy carriers, and using biogas technologies will also reduce emissions of methane gas from 'natural' (i.e. uncontrolled) decomposition processes of organic matter. As methane is particularly harmful as a greenhouse gas, biogas thus will reduce greenhouse gas emissions.

Biogas production also reduces unpleasant odours in agriculture in the vicinity of populated areas. Moreover, the residual biomass possesses an improved fertiliser quality and can be re-distributed in the fields more accurately than solid fertiliser or untreated organic waste. Of course, efficient fertilising reduces agrochemical pollution into surface and ground waters (Schulz & Eder 2001; Amon & Lindworsky 1998).

Biogas has also social benefits. Its production will generate alternative sources of income especially in the agricultural sector of poorer countries and for smaller local companies. This is of particular significance in regions that are remote or economically underdeveloped. Additionally, strengthening traditional forms of agriculture even has a socio-cultural dimension, as typical cultural landscapes may be preserved, even if new plants are introduced and new products are harvested. The opportunity to switch from low income agriculture to biogas production may convince small farmers to maintain in their business and by that preserve the identity of agricultural communities. Overall, biogas is a promising alternative for the future, because its resource base is widely available, and single farms or local cooperatives might start biogas plant operation.

Production base for energy plants - However, the production of bioenergy from food and forage crops has several critical disadvantages. Harvesting food crops for biogas production is not sustainable and not cost effective, as the valuable plant product that has been bred for decades to yield optimum nutrient content for food and feed, and not necessarily for fermentable biomass, is directly converted to gas and then end-oxidized at a relatively low economic yield. Furthermore, the biofuel production from food and fodder plants reduces the available amount of agricultural area, thus increasing food and fodder prices. Moreover, poorer countries and less developed agricultural regions will suffer significantly from this shortcoming, and they will have even less chances to develop sustainable production systems. Especially in the Mediterranean, agricultural production is limited by water availability and soil quality. The lack of financial resources on the farms leads to neglectance of the production base, the soil, and finally to soil degradation. These areas are usually abandoned and set aside; they are not available for food production. It turns out that the present development is at the verge to become non-sustainable, similar to our previous behaviour. The only way out of this dilemma is breeding novel plants for energy production that are able to grow in so far unexploited soils, enhance their capacity to build up biomass, and provide them with resistance against pests.



Figure 1: fallow land in a highly productive agricultural area in Germany



Figure 2: View of an agricultural region of northern Greece. Different soil colors depict fertile and infertile areas. Abandoned land with eroded and low organic matter soil is more obvious around the area closest to the photographer.

Taking this into account, fast breeding methods must be employed to yield novel plants with traits needed in the bioenergy industry, and abandoned marginal and degraded agricultural land must be used for their production. Promising species have to be tested for their suitability to produce readily digestible carbohydrate, protein and oil, and to be cropped in rotation with legumes and local (weed) species. These monocultures of novel plants will require novel plant protection measures as well as crop rotation protocols to ensure sustainable productivity. When farmers will grow novel energy plants in crop rotation with local plants and leguminous species, they will start to enlarge the local production base. After harvest, the biomass will feed biogas production in the dry fermentation process. The electricity system of the local community will be stabilized at low cost, and the process heat can be used for heating. Consequently, the digested solids will be used to ameliorate the land and improve the soil quality. Such knowledge based biotechnologies will provide the farming community with an opportunity to increase energy and food availability, to reduce energy costs and increase quality of life, to conserve fossil fuel reserves, fight climate change and create novel economic opportunities for small farms.

What biosciences should provide us with:

Provide tools to select and breed novel suitable energy plants for biogas production in a dry fermentation process. These plants can be grown in marginal lands and other unexploited soils of south eastern Europe. Specific crop rotation programmes will be set up and assisted by remote sensing, and rhizosphere processes will be considered to enhance plant performance and reduce plant stress. Tailor made fermentation processes will be designed for the crops and regions under study. Economical, ecological and social factors of the regions under consideration will also be taken into consideration.

Plant breeding options

Selection of desirable traits from conventional mendelian breeding under field conditions takes several years and many generations are needed to accomplish the goal. Conventional mutagenesis and *in vitro* selection can considerably shorten the time for the selection and can complement field selection. It is therefore an often successfully used fast-breeding approach with a proven experience of more than 40 years. With view to legal and societal restrictions, conventional breeding (such as mutagenesis or *in vitro* breeding) could be a promising alternative to genetic transformation for an improvement of yield and other traits of high yielding crops. Those classical non-GM techniques are free from safety regulations in the EU Member States, and will allow to screen & develop mutants in the greenhouse and under field conditions.

Mutagenesis - Mutation techniques have often been used to improve yield, oil quality, disease, salt and pest resistance in crops, or to increase the attractiveness of flowers and ornamental plants. In some economically important crops (e.g. barley, durum, wheat and cotton) mutant varieties nowadays occupy the majority of cultivated areas in many countries (Maluszynski et al., 1995). In the last 40 years, mutagenesis has also played an important role in improving agronomic characteristics of *Helianthus annuus* L., one of the most important oil seed crops in the world. Osorio et al. (1995) have reported an increased variability in the oil fatty acid composition of sunflower mutants, obtained from the seeds mutagenised with ethylmethanesulphonate (EMS). New sunflower mutants with an enhanced biomass production and oil content were obtained by Chandrappa (1982) after mutagenesis with EMS or DES. Kübler (1984) has obtained sunflower mutants of M₂ and M₃ generations with high

linoleic acid content for diet food and mutants with high oleic acid content for special purposes like frying oils after EMS mutagenesis. Nehnevajova et al. (2007) used chemical mutagenesis to improve yield and metal uptake of sunflowers. In the second mutant generation they have obtained sunflowers with improved yield (6-9 x), metal accumulation (2-3 x) and metal extraction efficiency (Cd 7.5 x, Zn 9.2 x; Pb 8.2 x) on a sewage sludge contaminated field. Whereas the best sunflower mutant could produce 26 t of dry matter yield per ha, the control sunflower inbred lines produced only 3 t dry matter per ha and year in the same experimental field. Based on these results it may be concluded that EMS mutagenesis mainly led to an enhanced biomass production with partially to improved metal accumulation in sunflowers.

Moreover, classical mutagenesis has also been used to get new mutant variants with reduced metal accumulation traits. Nawrot et al. (2001) have obtained barley mutants with an increased level of tolerance to Al toxicity. Ma et al. (2002) have shown a rice mutant defective in Si uptake obtained by a mutagenic treatment with sodium azide or Navarro et al. (1999) have isolated mutants of *Arabidopsis thaliana*, *cdht 1* and *cdht4*, which accumulated 2.3 less Cd than control plants exposed to 150 μ M CdCl₂.

Mutation breeding has a long history with cotton where newly developed genotypes occupy significant cultivated area in several countries (Maluszynski et al., 1995). Initially the efforts were concentrated towards improving either fibre quality or the pest resistance of the crop. With the more and more widespread use of cotton oil for dietary purposes interest has increased in modifying its fatty acid composition inducing variations through mutation (Abo-Hegazi, Shaheen et al. 1991; Bhat and Dani, 1993). However no attempts have yet been made to improve the energetic value of the plant with the view of biofuel production.

Several recent attempts have been made to characterize cotton biomass for its energy production characteristics, but those were mainly targeted towards the use either directly for heat production (Liao Cuiping et al., 2004) or for biodiesel production through catalytic pyrolysis (Ersan Putun et al., 2006). Mutation breeding will therefore add to the possible uses of the crop yet another option for making its cultivation profitable on poor, degraded and/or polluted soils. This will be supported by the growing interest in cultivating the crop in southern Europe, where the expansion in the last decade aimed mainly towards low-input cropping systems. The development of new cotton mutants, better adapted for biogas production under low-input cropping systems, could provide a new pathway in the use of this

traditional crop outside its much more obvious but much more resource-hungry use for fibre production.

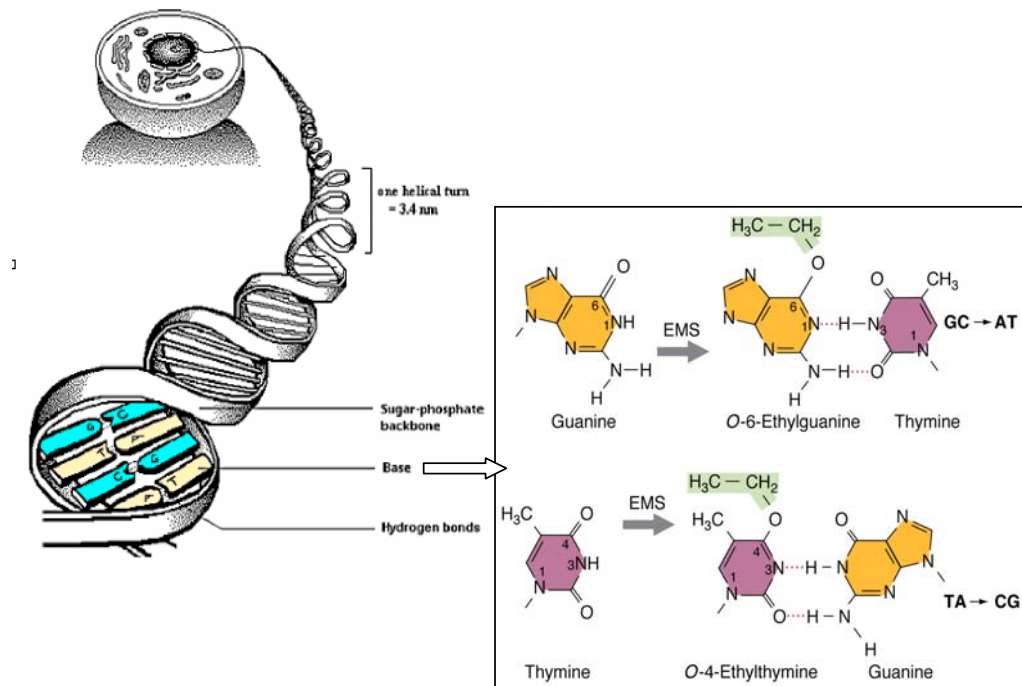


Figure 3: Chemical mutagenesis with ethyl metanesulfonate (EMS) as a promising approach for the production of novel plant varieties. EMS alters bases of the DNA by alkylating individual segments which leads to mispairings

In vitro breeding (somaclonal variation – spontaneous mutations) - Plant biotechnology involving modern tissue culture, using somaclonal variation and *in vitro* selection, offers the opportunity to develop new germplasm, better adapted to the changing demands (Alibert et al., 1994). Somaclonal variation can result in a range of genetically stable variations similar to the variations induced with chemical or physical mutagens (Skirvin et al., 1993; Jain et al., 1998). Somaclonal variation and *in vitro* selection seem to be also appropriate technologies for development of new plant variants with enhanced survival under adverse conditions (Herzig et al., 1997; Guadagnini et al., 1999; Nehnevajova et al., 2006; Jan et al., 1997; Ramgareeb et al., 1999; Sibov et al., 1999).

Fast breeding using molecular markers - Molecular markers are capable of providing efficient means for streamlining and speeding the breeding process. However such markers have never been used for selecting for traits related to biogas production. Within the frame of

the present project an attempt will be made to identify such markers in a model plant, well adapted to the conditions of the South-East Europe. Cotton was selected for this purpose as a crop that combines several essential characteristics for such kind of work to succeed: 1. irradiation mutagenesis is well known to induce large numbers of genetic modifications in a single treatment, thus increasing the chances that the traits, related to improved biogas production will be affected; 2. the identification of the most appropriate techniques and tools has already been a major subject of research (Bojinov and Lacape, 2003; Lacape et al., 2003; Bojinov and Bozhinov, 2004) and 3. Irradiation mutagenesis is well known to induce large numbers of genetic modifications in a single treatment, thus increasing the chances that the traits, related to improved biogas production will be affected. The identification of DNA-based markers, linked to improved biomass characteristics for biogas production, bears the potential to tremendously facilitate future breeding programs, aiming at developing specialized varieties for biogas production.

Helping plants to do the job

Identification of stress resistance of newly bred mutants - The molecular and biochemical mechanisms of plant adaptation to stress conditions are still poorly understood and the signaling pathways involved remain unclear (Dat et al, 2000, Schröder 2001). However, adaptation of plants to environmental changes is crucial for plant growth and survival. When growing energy crops on marginal land in a low input system, crops may suffer from various stresses such as drought, heat, pathogen attack, nutrient deficiency or metal toxicity. In recent years, reactive oxygen species (ROS) have been suggested to act as intermediate signalling molecules to regulate the expression of genes (Mittler, 2002; Mittler et al., 2004 Messner and Schröder 1999, Schröder et al. 2001). In this function, ROS have been proposed as a central component of plant adaptation to both biotic and abiotic stresses (Dat et al., 2000). These ROS are partially reduced forms of atmospheric oxygen ($^3\text{O}_2$) and can be very toxic since they attack several cell constituents such as proteins, lipids, or nucleic acids (Foyer and Noctor, 2005). Many stresses, including drought, metals, salt stress, heat shock or pathogen attack enhance the production of ROS (Mittler, 2002). The use of ROS as signalling molecules suggests that during the course of evolution plants were able to achieve a high degree of control over ROS levels. The steady state level of ROS in the different cellular compartments is therefore controlled by an interplay between different ROS-producing and ROS-scavenging mechanisms (including enzymatic and non-enzymatic reactions). Oxidative stress and

oxidative damage occur when there is a strong imbalance in the plant between both types of mechanisms.

Several studies have demonstrated effects on oxidative stress related responses (e.g. ROS-scavenging enzymes such as SOD, APOD, CAT, GST, GPOD, enzymes of ascorbate-glutathione pathway,...) and on other stress or housekeeping proteins (e.g dehydrin, actin, histone) in sunflower or tobacco exposed to drought, heat shock or metals at the metabolomic level (Bueno et al., 2002; Gallego et al., 1996; Synkova and Valcke, 2001; Zhang and Kirkham, 1996), as well as at the transcriptomic level (Kiani et al., 2007; Rizhsky et al., 2002). It is clear that oxidative stress related responses will influence plant performance and eventually lead to accumulation of unwanted phenolic metabolites in the plant material. Hence, differences in stress resistance of various plant genotypes have to be evaluated with specific emphasis on the differences between wild types and mutants, after exposure to increasing stress levels (drought, heat shock and/or metals).

Promoting plant growth by rhizosphere processes - The plant genome is often considered to completely regulate plant productivity and stress resistance. However, mutualistic micro-organisms have the potential to affect plant productivity and even stress resistance in some case. N₂-fixing rhizobia live in association with the roots in specialized structures (nodules) and provide nitrogen to their host. Due to this characteristic, leguminous cultures can help to enrich the poor soil with nitrogen, a property which makes this crop very suitable to take part in the rotation schemes of energy crops on marginal land. The potential beneficial effects of plant-microbe interactions on plant growth and tolerance are well documented by a variety of studies (Hall, 2002; Herrera et al., 1993; Mastretta et al., 2006). Mycorrhiza, as well as plant associated bacteria (rhizosphere or endophytic), can contribute to the nutrient supply of their plant hosts, which is important for optimal plant growth in normal conditions, but also for improving plant survival in hostile environments (Requena et al., 1997). Previous research with tobacco has already demonstrated that growth increases could be obtained (in laboratory experiments), after inoculation with selected bacterial strains. Exploitation of the possibilities of microbial associations therefore opens new perspectives for the improvement of yield and the increase of stress tolerance of energy crops grown on marginal land. Endophytic bacteria have previously been isolated from tobacco plants and seeds grown on metal contaminated soil in Northern Europe (Mastretta, 2007). Reinoculation experiments showed that positive effects on growth were observed after reinoculation of (sterile) plants with seed endophytes, while endophytes isolated from roots, stem or leaves on the contrary caused growth reductions (Mastretta, 2007). Mechanisms by which endophytic bacteria can promote plant

growth include e.g. biological nitrogen fixation, synthesis of (or influences on) phytohormones, or more particular properties such as metal tolerance or the presence of contaminant degradation pathways (Lodewyckx et al., 2001, 2002; Mastretta et al., 2006; Barac et al., 2004). An exploitation of such microbial associations, as proposed in the present project, opens perspectives for yield improvement and the increase of stress tolerance of energy crops grown on marginal land. Characterizing novel plants in this way will allow not only to conclude about the stress resistance of the various genotypes under investigation, but also to gain more insight in the (sequence of) events occurring in plants in response to environmental changes, and how are these regulated.

Combined isotopic measurements - The natural abundance of the stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in plant tissues has been used as integrated indicators of water stress and N nutrition, respectively. In the case of ^{13}C , the isotopic signal has been generally used to identify differences in water stress over large topographic gradients or between mutants. For estimation of productivity potentials (Laitha and Marshall 1009), in the case of $\delta^{15}\text{N}$, the isotopic signal has been used to identify N uptake of the source fertilizer (Bort et al. 1998). Recent evidence indicates that the combined isotopic signal in the leaves is sensitive enough to identify water- or nutrient-stressed plants over short distances within single fields. This finding is significant when considering that conventional analytical methods may be unable to detect such small spatial differences in plant stress or nutrient deficiencies. This novel approach of interpreting isotopic data will be an important tool for exploring the causes of biomass variation in the cultivation of energy plants.

Best practice in agronomy

Monocultures of bioenergy crops bear the danger of not being successful due to infertile soils, drought, pest or pathogen attack. All forms of agriculture cause changes in the balances and fluxes of pre-existing ecosystems, thereby limiting resiliency functions. The intensive agriculture in some regions, with its strong reduction of landscape structures and vast decoupling of energy and matter cycles, has caused stress and degradation of the production base; massive influence has also been exerted on neighbouring compartments. To overcome the economic, social and political inadequacies leading to ecological degradation during energy plant production, it is of paramount importance to transpose available knowledge on sustainable production systems, including remote sensing technologies, into knowledge-based practical instructions and political regulations on a regional scale. Thus, applied research for a

sustainable and ecologically compatible land use aimed at sufficient food production is ever so important (Schröder et al. 2004).

Proximal remote sensing - Studies with remote sensing have demonstrated the relationship between canopy spectral reflectance and biomass production of some major crops (Pinter et al. 2003). Reflectance data are typically derived from aircraft or satellite images in the visible and NIR ranges of the spectrum and subsequently transformed into vegetation indices (i.e. NDVI). However the availability of this information by airborne platforms is constrained by weather conditions, revisit frequency and elaborate data processing. On the other hand, proximal sensors are an emerging technology designed to overcome the limitations of current instrumentation by delivering real-time data of high spatial resolution that can be used in site specific management. Proximal sensors were recently used successfully for the prediction of biomass in irrigated corn, cotton and vineyards (Bausch and Delgado 2003; Stamatiadis et al. 2004, 2007). The application of this technology is expected to provide valuable management tools for increasing biomass production of energy crops.

Agricultural production systems have to be chosen that diminish soil and fertility losses, ensure water availability and fight pathogens. Farm practices that prevent erosion will help to protect soils and water quality. Sustainable agriculture will be achieved only when information about heterogeneity of soil-plant systems is available and appropriate plans are developed and implemented at the level of the local farm and the region. In order to make the production of novel energy plants possible, it is of utmost importance to develop adequate management systems. These should lower production costs, reduce pollution of surface and groundwater, reduce pesticide residues in food, reduce a farmer's overall risk, and increase both short- and long-term farm profitability (Parr et al. 1990).

One important trait of mutants intended for low-input systems of south-eastern Europe is their stress resistance to water shortage. Water stress will not only induce stomatal closure and limit photosynthetic activity, but also limit mineral N uptake and crop growth. The interaction between water availability and nutrient uptake is a crucial element that needs to be taken into account when testing the ability of new mutants to adapt to water-deficient environments. This is achieved in field plot experiments that combine different irrigation and fertilization rates in a split-plot experimental design.

Producing biogas

With fossil based fuel prices steadily rising as the resources diminish, bioenergy will grow steadily and is fast becoming an energy industry factor, currently accounting for around 3 percent of US energy production. By 2030 it should have reached more than 10 % in Europe. Moreover, as the EU and other countries stimulate research on energy production by legislation and corresponding incentives supporting Green Energy, investments into biogas production will become safe ground for agricultural communities. Modern dry fermentation has processing and technical advantages over the liquid fermentation process currently used for producing biogas.

Reactor types - State of the art digestion systems for energy crops are so called **aqueous** digesters, i.e. the energy crops are shredded and fed into the digester with a large amount of additional water leading to a concentration of usually below 5% dry substance in the reactor. This leads to large volumes of the digestion vessels since the residence time needs to be held at approx. 40 days in order to fully convert the digestible organic matter. The reactor type is – according to text book – an agitated vessel and thus operates always at minimum or final concentration of organic matter.

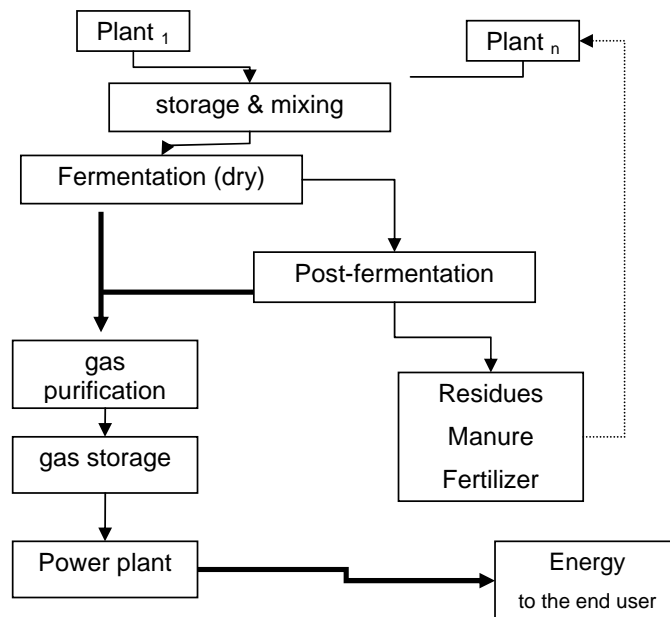


Figure 4: flow chart of the biogas production process

Newer technology – so called **dry digestion**, does not dilute the organic matter with water but keeps the organic matter unmixed with already digested matter and thus keeps the organics concentration always as high as possible. Using such a **plug flow** type reactor results in lower volumes due to the much higher concentration of organics which range from approx. 35% to 12% between the feeding stage and the final stage of the process and the shorter residence time resulting. An intercomparison shows that the **plug flow** type is very successful for organic waste applications because of its rigidity against inhibition and side reactions. However, for energy crops there is only little experience with this reactor type.

Research needs - The energetic quality of the biogas depends on the fermentation process and on the characteristics of the actual substrate fed into the reactor. The challenge of optimal biogas production is to maintain a stable anaerobic digestion process. Technical solutions for this task are limited. Instead, it requires good quality energy plant material, accuracy, perseverance and experience on the side of the plant operators. Technical protocols tackling these problems are lacking. The basic substrate for biogas production should be organic substances derived from plant matter, although other organic wastes might be added to the reactor. Residues of vegetable or animal fat are of particular interest in this respect, as they have high calorific values and thus enable high biogas yields. This co-digestion might also be beneficial for stabilising the fermentation process. It is known that inhibiting reactions may be caused by side products of the reactions or by ingredients originating from the crops. Particularly difficult is mono digestions due to lack of trace minerals and – in the dry digestion – the possibility of high salt concentration leading to high osmotic pressures which might kill the bacteria or at least seriously damage them. Using new energy crops may therefore lead to unexpected problems in the process which might lower the gas production rates as compared to an industrial environment. Hence it is crucial to scrutinize the digestion process for the new crops, because otherwise, if digestion is deteriorated by unexpected metabolites, new crops may not be useful although they seem promising at first glance.

Conclusions

In the European Union, biomass is regarded the most promising renewable energy option for the closer future (European Commission 1997; Wellinger *et al.* 1991). Biogas in particular has received considerable attention as a renewable energy carrier with a huge potential for the European market. Being usually produced by anaerobic digestion of wet agricultural products (including manure), the most widespread use of biogas is seen in the decentralised generation of electricity and heat to cover local demand. Excess electricity can even be fed into local

electricity networks. Alternatively, biogas can be fed into the natural gas grid, for power generation or heating purposes, or as a vehicle fuel. Biogas reactors are mostly situated on farms to support local consumers. Biogas potential has been exploited to various extents and in different ways in different countries and regions. In Austria, Germany and Switzerland numerous biogas plants have been established, and the United Kingdom, France, the Netherlands, Denmark, Sweden and Belgium are also starting to further this technology. However, most southern European countries, and especially new EU member states have not adopted this promising way to improve their local energy production. Now it is necessary to evaluate the potential of abandoned soils in south eastern Europe to grow plants that produce sufficient biomass at low costs to present a sustainable energy supply to poorer regions in Europe. Even the 7th Framework takes this lack of knowledge into account: Although high-tech solutions for growth of biomass are presently available all over Europe, their sustainability is usually not achieved, resilience to numerous parameters is questionable, and clearcut evidence is presented in EU papers that these technical solutions are too expensive for many communities.

Thus, besides scientific progress, it will be of high importance to strengthen the European Research Area (ERA) by international cooperation to achieve common and well developed strategies that respond to global change issues of high public concern such as the energy plant and biogas production. These strategies should be applicable in all member states, not in the least to those with lower financial means. Cost effectiveness, reliability, long-term sustainability, resilience and reasonable input of resources are key characteristics of the present approach. To achieve these goals, scientific and practical developments are needed. Breeding new plants by a fast-track-breeding approach based on mutagenesis, aiming at the development of new genotypes which are particularly adapted to marginal soils, and resistant to the extreme climatic conditions in southern Europe is one of the most promising alternatives to conventional breeding, but also to GMO approaches. The exploitation of plant-microbial interactions as an alternative/complement to chemical fertilisers to improve yield of plants is well known in organic farming, and it should be applied in this novel field of energy plant production, as well as the evaluation of the stress resistance of new plants by metabolomic and transcriptomic analyses.

Further, and to not fall back into the mistakes of the agricultural production in the 1970ies and 1980ies, it will be important to optimize rotation schemes and soil management practices, hand in hand with an optimisation of crop mixtures in bio-reactor for optimal energy production. And last not least, the operation of biogas production units with the novel plant

material, and testing bacterial consortia for their suitability in fermentation will demonstrate the feasibility and good practise to local stakeholders.

Europe is one of the leading players in the advancement of technologies and related environmental applications. European remote sensing satellites cover all of the Earth's climatic zones, while European ground-based, air-based and ocean-based monitoring devices serve users by providing high quality observation data in areas as diverse as urban planning, adaptation to and mitigation of climate change, disaster reduction, disease control and humanitarian relief. Research on bio-energy plants and fossil fuel reduction is one of the major aims for the next decade, and this can not be achieved by small groups or single institutions. With view to the impact in our information society, it is necessary to include as many stake-holders and groupings as possible in successful developments. Through the European Union's exhaustive efforts to promote greater awareness of science in society, results emanating from its environmental research initiatives may also have knock-on effects on the day-to-day decisions made by industry, commerce and Europe's citizens – the ultimate goal being to find a sustainable balance. Especially teams that include scientists from associated countries or new member states will influence this development by working together and investigate burning topics, with the aim of producing new environmental tools and technologies to promote sustainable development. This will help to establish rules and regulations for biogas production in the EU core countries and in the countries that will enlarge the ERA, and they will raise interest for the technologies by improving public awareness of the green technology used to protect our most valuable resource, the climate. Especially involving SME partners will result in an enlarged cooperation and technology transfer across Europe, thus confirming the role of Europe as a leader in the export of innovative and environmentally friendly environmental technologies at affordable costs and high efficiency worldwide.

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