toward sustainable paradigms that either are energy-neutral or output energy, and therefore the main-stream deammonification-based process is the more promising technology. The DAMO–ANAMMOX reactor would be at the heart of mainstream deammonification (Figure 2B, solid line). First, most of the organic matter (~80%) and phosphorus (~90%) in wastewater is absorbed and stored in the high-rate activated sludge-based bioreactor [14]. The ammonium-rich effluent is then treated in a nitritation reactor to partially convert ammonium to nitrite [15]. Finally, the nitritation effluent is further treated in the DAMO–ANAMMOX reactor. Using this approach, the DAMO-based technologies have significant potential for the sustainable operation of WWTPs.

The Way Forward

To make DAMO suitable for field application, the following issues should be addressed in the future.

(i) Further understanding the microbial behaviors of DAMO microorganisms. To accelerate the cultivation period of DAMO microorganisms and increase reaction rates, efforts will be necessary to further elucidate the physiology, mechanisms, and kinetics of the known M. oxyfera and M. nitroreducens species, identify other microbes potentially carrying out DAMO process, and explore the interaction between DAMO microorganisms and other microbial groups.

(ii) Scaling up DAMO to a practical level. The biggest challenge is how to concurrently scale up the system size to a real-world level while achieving practically useful reaction rates and performances. In addition, the gas generated by the digester also contains other components such as CO_2 and H_2S, and thus their impact on DAMO needs to be considered in field situations.

(iii) Better controlling the DAMO hybrid process. In engineered systems, reasonable coordination between DAMO and other units such as activated sludge unit and nitritation is crucial for the successful application of such emerging technology. Such a combined system requires delicate optimization of both system design and process operation, and mathematical modeling and advanced monitoring/control strategies may be useful in this regard. However, this could increase the construction investment, a factor that will need to be evaluated in the future.

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Special Issue: Environmental Biotechnology

Science & Society

Ethnophytotechnology: Harnessing the Power of Ethnobotany with Biotechnology

John de la Parra[1] and Cassandra L. Quave[2,3,*,@]

Ethnobotany (the scientific study of traditional plant knowledge) has aided the discovery of important medicines. However, as single-molecule drugs or synergistic mixtures, these remedies have faced obstacles in production and analysis. Now, advances in bioreactor technology, metabolic engineering, and analytical instrumentation are improving the production, manipulation, and scientific understanding of such remedies.

Table 1. Advances in Ethnophytotechnology[a]

Plant species	Ethnobotanical uses	Modern medicinal component	Relevant biotechnology interfaces	Refs
Atropa belladonna	Dilation of pupils, cosmetic	Scopolamine	TC, ME, BT	[4,5]
Artemisia annua	Fever remedy	Artemisinin, additional synergistic compounds	TC, ME, BT	[3–7]
Catharanthus roseus	Diabetes, fever remedy	Vincristine/vinblastine and other alkaloids	TC, HE, ME, BT, AB	[2–5,7]
Papaver somniferum	Pain management, soporific, cough remedy	Morphine and other alkaloids	TC, ME, BT, AB	[3,4,7–9]
Podophyllum peltatum	Warts, topical skin ailment remedy	Podophyllotoxin, mayapple etoposides	TC, ME, BT, AB	[2,4,5,7,9,10]
Taxus brevifolia	Wound healing, lung issues, religious uses	Paclitaxel	TC, ME, BT	[2–5,7]

[a]Abbreviations: AB, analytical biotechnology; BT, bioreactor technology; HE, hormonal elicitation; ME, metabolic engineering; TC, tissue culture.

The Origins of Ethnobotany and Plant Biotechnology Interfaces

Until approximately 40 years ago, most primitive plant 'biotechnology' occurred in the form of selective breeding and was undertaken by indigenous cultures carefully choosing favorable phenotypes of economically valuable plants [1]. The field of ethnobotany helps describe this ancient practice and, from those studies, we can often gather how, when, and where specific plants were chosen for food, construction, or even distinct medicinal properties [2]. Although our ancestors were concerned with improving all of their crop types, modern-day plant biotechnology is most often associated with the engineering of high-profile agricultural innovations, such as Roundup Ready® corn and Golden Rice. While these products have been recognized for spurring economic growth and impacting human health, similar advances in the field of drug discovery and production from medicinal plants have been less prominent. Besides economic factors, this has been due, in part, to scientific challenges that have confronted traditionally used ethnobotanical medicines. Their biosynthesis is often supremely complex, low yield, and not well understood. Their mechanisms of action are often unknown or seemingly reliant upon the synergistic interactions of many compounds in an extract.

However, emerging production, engineering, and analysis technologies from the world of plant biotechnology are making these challenges easier to meet and, therefore, stand to revitalize interest in ethnobotanical research. Thousands of years of indigenous knowledge can find prominence in modern drug discovery and production platforms at a time when the rise of drug-resistant microbes and cancers has already driven exciting work in botanically derived pharmaceuticals [3]. These advances and pressures have encouraged the dawning of an era of ethnophytotechnology: the use of plant biotechnology to improve or enhance the inherent economic or culturally valuable traits of plants as described and influenced by ethnobotany. For this emerging interdisciplinary practice in drug discovery and production, we are witnessing the integration of ancient and/or contemporary ethnobotanically inspired investigations with innovative biotechnological manipulation and analysis (Table 1).

Bioreactors, Tissue Culture, and Hormonal Elicitation

A primary question arises for many ethnophytotechnologists: an indigenous healer may pick a leaf or root from the forest at a specific place and time for a precise therapeutic action, but how do they guarantee that they can go back tomorrow and get the same results? Researchers interested in ethnobotanically derived medicines have often been hampered by this irreproducibility as well as by low yields of bioactive compounds. However, similar to the way that engineering advances in the improvement of bioreactor fermentation processes allowed for the mass production of penicillin, plant-extracted pharmaceuticals are now on track to benefit from a synthesis of technological progress. Large-scale bioreactors are being designed specifically for the standardized and controllable production of botanical drugs. Unique plant cell and tissue cultures have been adapted for use in bioreactor technologies to deliver the reproducibility and enhancement of active component production [5].

It is possible that indigenous knowledge could enhance production even further. By analyzing ethnobotanical studies to glean the timing and conditions of plant selection (i.e., presence of biotic or abiotic stressors), valuable chemical ecology insights may be the next clue to increased drug production. For instance, when a plant is under attack, it produces endogenous hormones that regulate the production of specialized defense compounds, what we might call drugs. By tinkering with the exogenous administration of hormonal elicitation, perhaps even in accordance with indigenous selection, meaningful increases in plant-derived drugs could be achieved [4].

Tissue culture biotechnology also opens up drug development opportunities for rare, endemic, or endangered species with limited natural population

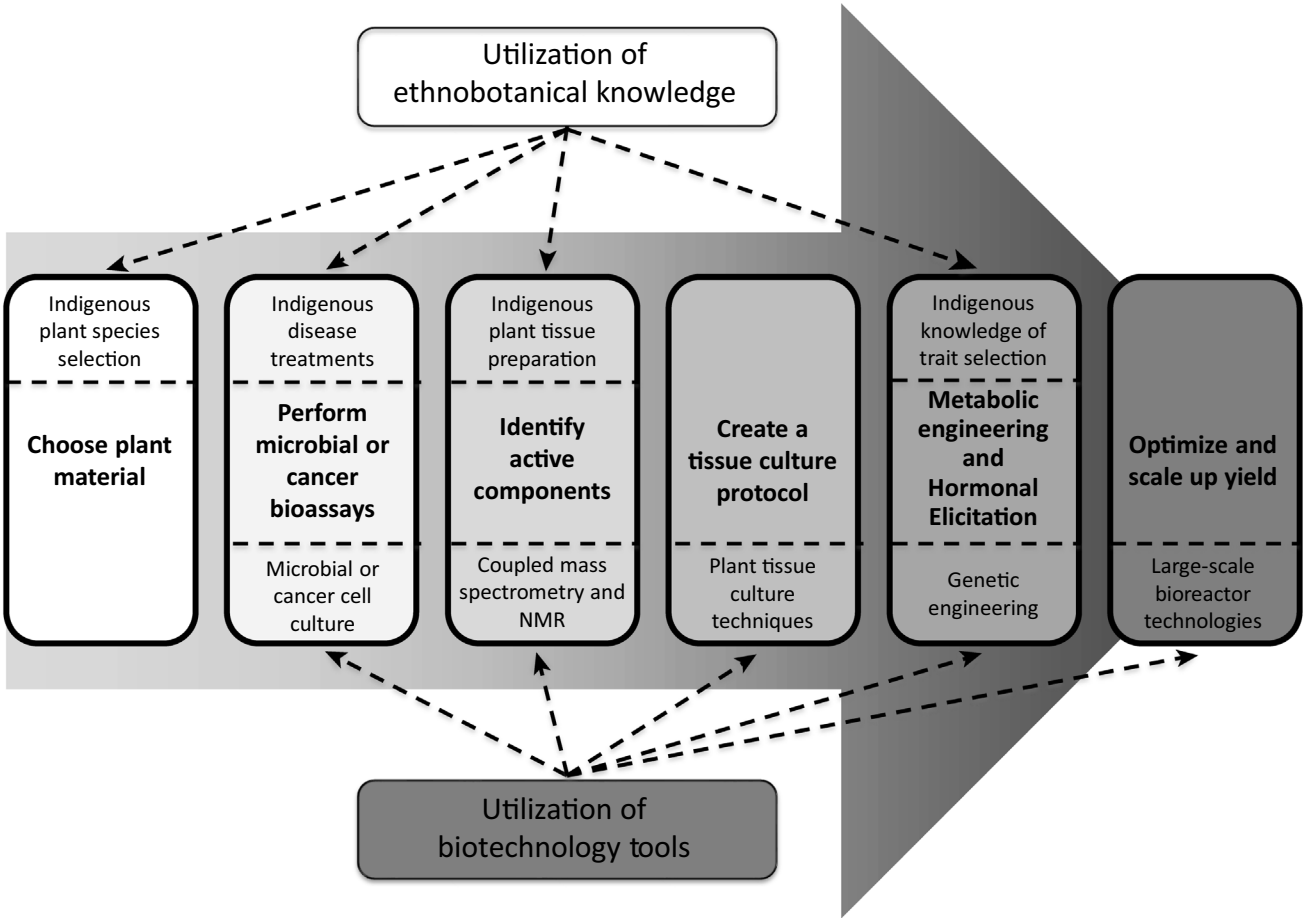

Figure 1. Proposed Example of an Ethnophytotechnology Workflow for Botanical Drug Improvement and Scale-Up. Workflows in ethnophytotechnology can take several forms, but generally rely on an integrated platform of enhancing the yield and analysis of ethnobotanically derived drugs with the tools of biotechnology and reciprocally informing plant biotechnology techniques with ethnobotanical information. In this example, ethnobotanical knowledge is used to identify novel pharmaceutical compounds or fractions that are then subject to analysis, engineered, and scaled up using the tools of biotechnology.

ranges and for which bulk collections would not be sustainable. It is often no small task to collect enough plant material to amass a particular low-yield bioactive compound for advanced analytical techniques and bioassays. However, with tissue culture, it becomes possible to grow plant cells in a bioreactor, administer hormones to spike and diversify metabolite levels, and then extract higher yields for further analysis.

Metabolic Engineering and Phenotyping

One of the most exciting biotechnological advances impacting traditional medicinal plants has been the engineering of specialized metabolism. Here resides a promise of solving the ethnobotanical problem of low-yield metabolites. These same methods could also improve the action of botanical drugs by altering enzymes to produce novel metabolite structures, even halogenated compounds [7]. Just as in crop plant engineering, the metabolic engineering of medicinal plant tissue can be almost exclusively facilitated through *Agrobacterium*-mediated gene transfer [11], although work with DNA-coated particle bombardment and CRISPR technologies is advancing [12]. Recent work to produce medicinal poppy-derived alkaloids has shown that this metabolic manipulation does not exclusively need to occur in plant cells, but rather entire metabolic pathways can be transferred to yeast for potential large-scale production [8]. The tools now exist to complete this work for other botanical drugs, but there are often numerous unidentified genes involved in these pathways – a bountiful source of bottlenecks for ethnophytotechnologists to explore [10].

While ethnobotany cannot provide immediate clues as to which enzymes or transcription factors might need to be regulated to target limiting steps in metabolite biosynthesis, we do get chemical clues from the anthropological record.

Trait selection is often an ethnobotanical cornerstone. The original medicinal plant phenotyping was likely undertaken by indigenous healers who would seek out not only a particular species of plant, but also a particular population of plants. The value of phenotyping has been echoed by the American Society of Plant Biologists, who have recently set new goals for progress in high-throughput plant phenotyping [13]. A dialog with ethnobotanists could lead to new insights by, for example, utilizing established big data techniques to catalog and database ethnobotanical trait selection for medicinal plants.

Analytical Biotechnology

One of the biggest challenges in ethnobotanical drug discovery and production has been active component identification from crude botanical extracts. Therefore, ethnophytotechnology begins and ends with high-quality analysis afforded by recent instrumentation advances. Liquid chromatography coupled with mass spectrometry has been invaluable for the preliminary identification of bioactive plant metabolites. This technology has helped expand the search for botanical pharmaceuticals from small molecules to bioactive peptides from traditional medicinal plants [14]. Analytical chemists are also rapidly improving methods to obtain spatiochemical information by passing plant tissue directly in front of a detector [9]. One can imagine a time when portable mass spectrometers might accompany ethnobotanists to the forest to scan leaves for interesting compounds [15]. In addition to mass spectrometry, NMR represents a critical step in structural identification. Advances in high-resolution instruments are affording chemists the ability to work with substantially reduced amounts of isolated material to achieve structural resolution. These techniques will be essential tools in the field of ethnophytotechnology.

Advances in analysis also give the ethnophytotechnologist the benefit of more accurately describing the multitude of potentially crucial components in complex plant extracts. Reductionists have been skeptical, often rightly so, of imprecise claims of botanical synergism or potentiation as a nonrigorous explanation of drug action. However, even with a well-known isolated botanical drug, such as artemisinin, the indigenously used whole-plant formulation has shown increased activity [6]. It is likely that our ability to analytically describe and reproduce those results will be enhanced by analytical ethnophytotechnologists.

Potential for an Integrated and Practical Workflow

Ethnobotany has always been an interdisciplinary endeavor that, by its very nature, evolves as the human capacity for technology evolves. In this way, plant biotechnology can be seen as a natural extension of ethnobotany: it is becoming one of the predominant ways that humans and plants interact in the modern world. However, ethnobotanists must value and consider implementing the tools of plant biotechnology. What could be a vast and expanding field has been viewed by many for the past few decades as esoteric. Ethnophytotechnology provides an opportunity for new scientific innovation, rigor, and revitalization. In addition, while words can help focus our intent to innovate in a specific manner, the real-world integration of disciplines is where scientific impact will be made. As practitioners, we are tasked with developing methods for an academic and industrial environment that value both ancient human plant knowledge and the advances that have come on the technological cutting edge.

There are many possibilities for ethnophytotechnology workflows that can be tailored to the ethnobotanical information and specific technologies available. However, a method often begins with anthropological data collected with careful adherence to international conventions, such as the Nagoya Protocol, which has been ratified by approximately 100 countries and provides guidance on how to fairly share profits gleaned from the genetic resources of indigenous people. One can then proceed to analyze that information rationally and provide empirical evidence for initial ethnobotanical claims. Analytical biotechnology speeds this process and provides peerless data to support moving to the standardization and reproducibility offered by tissue culture. All of these methods have been utilized with great success in distinct fields, but here we have integrated them under a cohesive scientific discipline that digests the ethnobotanical evidence into testable hypotheses within the world of plant biotechnology (Figure 1). Ethnophytotechnology brings the tools of modern plant biotechnology manipulations to bear on a long ethnobotanical legacy, leading to precise analysis and large-scale production of pharmaceuticals that may result in life-saving treatments.

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Special Issue: Environmental Biotechnology

Forum

Carbon Concentration in Algae: Reducing CO_2 From Exhaust Gas

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Algal carbon-concentrating mechanisms can be used to sequester CO_2 from the atmosphere, and the resulting biomass can produce various value-added products. Mechanisms for carbon concentration in algae are complex and sometimes inefficient. We need to understand how algae successfully overcome these challenges while capturing CO_2 from their nearby environment.

Exploring Algal CCMs: A Green Path Toward Carbon Capture

Microalgal and cyanobacterial carbon-concentrating mechanisms (CCMs) are the most extensively studied aquatic CCMs. However, these microorganisms encounter a number of challenges in capturing CO_2 from their nearby environment. One challenge is that the enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco) possesses very low affinity for CO_2 and is an extremely slow enzyme, working at only about 25% of its catalytic efficiency. In addition, in an aqueous medium, CO_2 diffuses 10 000-fold more slowly than it diffuses in air [1]. Furthermore, oxygen can compete with CO_2 for fixation by Rubisco. One of the products of the oxygenase reaction, 2-phosphoglycolate, results in the loss of CO_2 because this phosphoglycolate is metabolized to glycine, which with another glycine molecule forms serine. This process, known as photorespiration, ultimately reduces the photosynthetic efficiency of Rubisco and diminishes photosynthetic carbon fixation [2]. Finally, the availability of inorganic carbon (Ci) either in the form of CO_2 or HCO_3^- depends on pH, and therefore there are wide variations in the availability of inorganic carbon for photosynthesis. When the pH is acidic, much of the Ci is present as CO_2, and when the pH becomes alkaline, most of the Ci is present as HCO_3^-.

Key players in both prokaryotic and eukaryotic algal CCMs include inorganic carbon transporters (Box 1) that derive energy from ATP, NADPH, or an ion gradient; and microcompartments that house Rubisco and carbonic anhydrases (Figure 1). Rubisco is localized inside carboxysomes (specialized protein microcompartments with polyhedral protein shells) in cyanobacteria, whereas in eukaryotic algae Rubisco is generally housed in single or multiple pyrenoids (proteinaceous components of chloroplasts that separate Rubisco from stroma) [3].

Several proton pumps present in both prokaryotic and eukaryotic algal cells help to maintain pH homeostasis on dehydration of bicarbonates [4]. With the help of inorganic carbon transporters, HCO_3^- is concentrated in the bulk cytoplasm, and the enzyme carbonic anhydrase converts this cytosolic pool of HCO_3^- into CO_2 within the carboxysome, resulting in increased local concentration of CO_2 around the active site of Rubisco [1]. The transport systems responsible for carbon concentration in cyanobacteria can be present at either the plasma membrane, also known as the plasmalemma, or the thylakoid membrane. Thylakoids in cyanobacteria are membrane-bound structures inside the cell and are the sites for light-dependent photosynthetic reactions.

Unraveling the Prospects for Algal Biofixation

Much effort has been made recently to study and understand CCMs in algae, but many components of microalgal CCMs are still unknown and not characterized. Some algal species are inefficient in inorganic carbon acquisition when grown under elevated levels of CO_2 (one study considered concentrations 10-fold higher than the ambient CO_2 concentration of ~400 ppm [5]), whereas when the same algal species are grown under low CO_2 levels they are able to uptake CO_2 relatively efficiently [1]. One explanation for this result is the presence of inducible transport mechanisms. Mutant studies involving screening of mutagenized cells for growth on high and low CO_2 would help in understanding the Ci uptake ability of the algal species and in characterizing novel proteins involved in CCMs [1]. Moreover, a much greater understanding is required about the mechanism that induces CO_2-responsive genes under Ci limiting conditions as well as the transcriptional regulation of various genes involved in CCM.

Coal-powered industries and fossil fuel-burning activities have led to enormous greenhouse gas emissions from exhaust gases. Algal carbon-concentrating mechanisms can be used as a tool to sequester CO_2 from the atmosphere because their biofixation efficiency is much greater than that of terrestrial plants. CO_2 as a source