

Acid catalyzed auto-hydrolysis of *Parthenium hysterophorus* L. for production of xylose for lignocellulosic ethanol.

Shubhaneel N¹, Swati Ghosh², S Haldar³, A Ganguly⁴ and P K Chatterjee^{4*}

¹ Periyar University, Salem, India

² Department of Chemistry, Institute of Engineering and Industrial Technology,
Durgapur – 713212, India

³ Department of Chemical Engineering, National Institute of Technology,
Durgapur – 713209, India

⁴ CSIR Central Mechanical Engineering Research Institute, Durgapur – 713209, India

¹shubhaneel@gmail.com, ²swatighosh0209@gmail.com, shaldar_nitd@yahoo.co.in
⁴amithort@rediffmail.com, ^{4*}pradipcmeri@gmail.com

Abstract— the obvious search for renewable & green biofuel, in respect to the definite future scarcity of natural petro-fuel directed to the use of lignocellulosic biomass as a promising future. The *Parthenium hysterophorus* L., having an excessive growth rate & adaptability, is showing a high prospect as a resource of rich lignocellulosic biomass available for fermentation. It is being ignored from a long time due to its toxicity characterized by the majority presence of Parthenin toxin, the potential growth inhibitor. Being a lignocellulosic biomass, with an estimated composition of lignin: 13.9%, hemicellulose: 21.01% and cellulose: 27.8%, it may be considered as the potential source of ethanol production substrate through pre-treatment, saccharification and fermentation pathway. The purpose of the present research work is to utilize the obnoxious parthenium weed to produce the next generation fuel substitute. There are mainly two processes involved in the conversion: hydrolysis of hemicellulose and cellulose of the lignocellulosic biomass to produce reducing sugars and fermentation of the sugars to ethanol. Lignin was removed initially by pre-treatment and hemicellulose can be converted to xylose, which synergistically with cellulose can be further converted into ethanol by simultaneous saccharification and fermentation process. In this work, efforts have been focused to increase the xylose yield during auto-hydrolysis. Hydrolysis of *Parthenium hysterophorus* was carried out with 1%, 3% & 5% H₂SO₄ solution. Each set of biomass was loaded with 10% of each acid solution against the mass taken and autoclaved for 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 8 min, 9 min, 10 min, 15 min, 20 min, 25 min, 30 min at 121 °C. The xylose yield has been studied for every set of parameters. The maximum value of xylose yield obtained is around 61.7 mg/g of dry *Parthenium*, when auto hydrolysed with 5% H₂SO₄ at a temperature of 121 °C.

Keywords— Auto hydrolysis; pre-treatment; lignin; hemicellulose; fermentation.

I. INTRODUCTION

The global shock with a drastic price hike of crude oil, 100 \$ per barrel in January 2008 [1], left a dark spot in face of industrialization questioning on the dependability & feasibility of crude oil in near future. It also was a symbolic indicator for the decreasing availability of conventional energy sources due to faster global economy growth. Particularly in less developed countries and newly industrializing economies like India and China. According to the World Energy, Technology, and Climate Policy Outlook of the European Commission,

there will be a 240 % raise in the energy consumption of Asia by 2030 (based on 2000). The world total energy consumption will rise from 9,927 million tons of oil equivalent (Mtoe) to 17,100 Mtoe in the same period (Figure 1.1). As a direct consequence, the world carbon dioxide emission will be almost doubled by 2030 (Figure 1.2) [2].

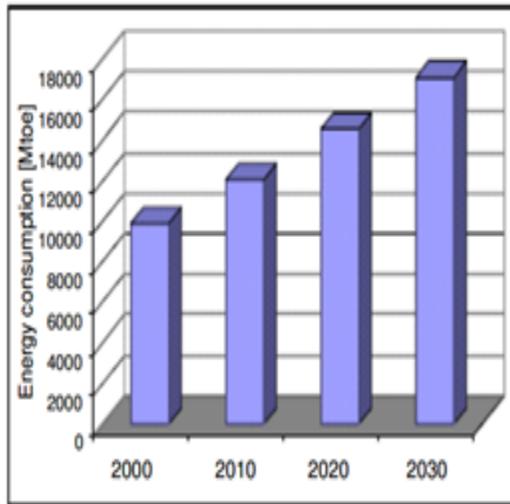


Fig 1: World total energy consumption [2].

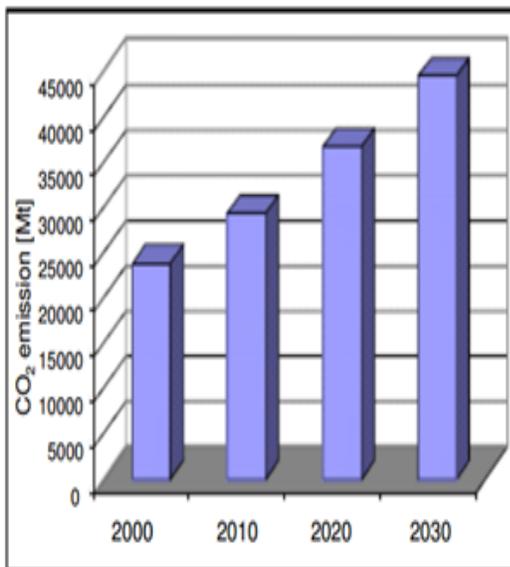


Fig 2: World CO₂ emission. [2]

So it became essential to find out alternative energy sources, in shake of the health of environment too. Here the utilization of bio-ethanol, the next generation green fuel, easily fermentable from the available sugar in lignocellulosic biomass is showing a bright aspect as a replacement of conventional fuel. It is being used as gasohol or oxygenated fuels since the 1980s.

Now a days there is an extensive focus on development of sustainable energy resources and if possible, by using waste resources to recycle it on an utilizable way. Here the lignocellulosic wastes from forestry, agricultural residue, yard waste, wood products

wastes, animal and human wastes, etc., can be utilized as a potential source of low-cost ethanol. In the last two decades a dedicated focus is in researching conversion of lignocellulosic materials to ethanol and its advancement were focused by a lot of researcher throughout the world (Dale et al., 1984; Wright, 1998; Azzam, 1989; Cadoche and Lopez, 1989; Reshamwala et al., 1995; Bjerre et al., 1996; Duff and Murray, 1996; Carlo N Hamelink et al.). The ligno-cellulosic conversion basically focuses on, hydrolysis of cellulose in the lingo-cellulosic materials to fermentable reducing sugars, and fermentation of the sugars to ethanol. Hydrolysis contributes to conversion of polysaccharides into monomeric sugars. The cellulose in ligno-cellulosic biomass, organized into micro fibrils, measuring about 3-6 nm in diameter and containing 36 glucan chains having thousands of glucan residue [3]. Cellulose is hydrolysable into glucose either enzymatically or by acids (e.g. H₂SO₄). Hemicellulose, a branched polymer composed of pentose and hexose sugars, can be hydrolysed by hemicellulases or acids to release its component sugars. Hexoses such as glucose, galactose, mannose are readily fermented to ethanol by many naturally occurring organisms (e.g. *S. cerevisiae*), but the pentose's including xylose, arabinose etc. are fermented to ethanol by few native strains, and usually at relatively low yields. Since pentose's comprises a significant fraction of lignocellulosic biomass, it must be utilised along with hexose to make the process economically feasible. The development of recombinant or GMO of bacteria & fungus are sometime capable of co-fermenting pentoses & hexoses into ethanol. The overall hydrolysis process of lignocellulosic biomass is greatly dependent on accessible surface area of the material, i.e., porosity, cellulose crystallinity, and lignin and hemicellulose content (McMillan, 1994). Therefore the main intensity of pre-treatment is to remove lignin, to breakdown hemicellulose into mono-sugars & to reduce cellulose crystallinity. So the design of pre-treatment phase require some attention on: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of inhibitory by-products to the subsequent hydrolysis & fermentation process; (4) in whichever mean, chemical, physico-chemical or biological processes, it have to be economically viable [3].

Parthenium hysterophorus, the species of flowering plant of Asteraceae family, i.e., native to the American tropics, also known as congress grass, carrot weed, star weed, feverfew, white top, chatak chandni, bitter weed, ramphool, garghas depending on the country and area infested. This weed is well known for its allergic responses to human being, causing respiratory problems, contact dermatitis, mutagenicity in human & livestock.

Biodiversity is greatly altered & crop production is remarkably reduced by its allelopathic properties, characterized by the presence of highly alkaline toxin perthenin of sesquiterpene-lactone group of compound (Singh et al. 2003*).

Parthenium hysterophorus is able to grow on a wide range of soil types ranging from sandy to heavy clays but growth is better in moist type of soil. It occurs in areas with summer rainfall greater than 500 mm per annum. Germination favours at temperatures between 10 °C and 25 °C. *Parthenium* weed colonises new areas rapidly by means of relatively high numbers of seeds, producing one lakh seeds per plant. Dispersal of seed takes place via vehicle, water, animals, farm machinery and wind. Disturbed habitats such as roadsides and railway tracks, stockyards, buildings surroundings and fallow agricultural land are particularly suitable for growing *P. hysterophorus* due to a lack of interspecies competition [4].

The waste land weed *parthenium hysterophorus* has been recorded growing and colonising naturally since centuries in Mexico, Cuba, North and South America, West Indies, Australia, Taiwan, Southern China, pacific island, East and South Africa, Asia, Oceania and Canada. Till 1977 the weed was not considered as the world's worst weed, but within the last decade it has become one of the seven most dreaded weeds of the world.

Parthenium weed was first noted in India near Poona in Maharashtra State in 1951. By 1972 it had spread into the most of the western States from Kashmir in the north to Kerala in the south and is now spreaded almost throughout the subcontinent with abnormal density [5].

Chemical analysis of *P. hysterophorus* has indicated that all its parts including trichomes and pollen contains toxins called perthenin, a major sesquiterpene lactone. Other allelochemicals are hysterin, ambrosin, flavonoids such as quercelagetin 3, 7-dimethyl ether ($C_{17}H_{14}O_8$), and fumaric acid ($C_4H_4O_4$), p-hydroxy benzoin (HOC_6H_4COOH) and vanillic acid ($C_8H_8O_4$), caffeic acid ($C_9H_8O_4$), p-courmaric acid ($C_9H_8O_3$), p-anisic acid ($C_8H_8O_3$), chlorogenic acid ($C_{16}H_{18}O_9$), ferulic acid ($C_{14}H_{14}O_4$), beta-sitosterol ($C_{29}H_{50}O$) [6] and some unidentified alcohols.

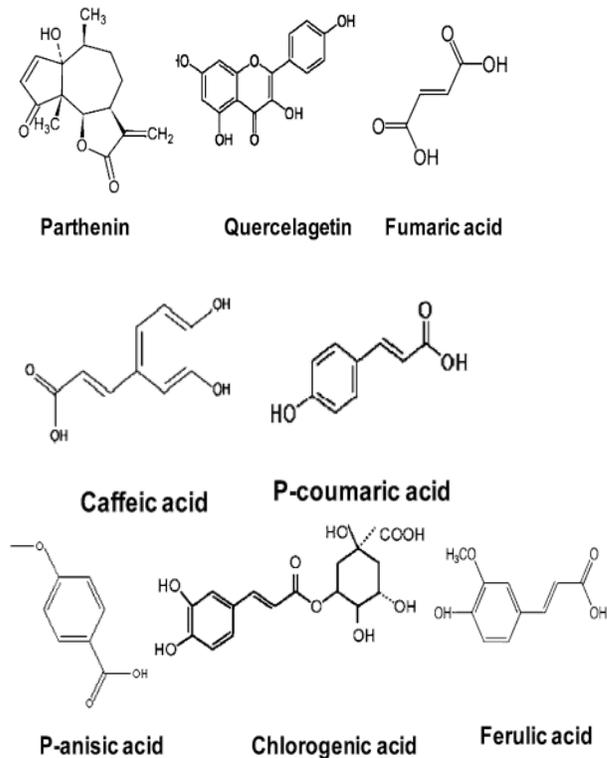
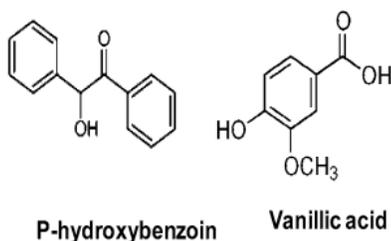


Fig 3: The Molecular structure of some of the major components of *Parthenium hysterophorus* L.

The main objective of this work is to apply a simple feasible pre-treatment process with moist heat dilute acid hydrolysis of the conversion of dry parthenium to release the xylose. The parthenium used to this study was previously estimated, having a composition of 27% cellulose, 21% hemicellulose, 12-15 % lignin as determined in the laboratory. Estimation of xylose which is a major fermentable pentose sugar, have been performed by spectrophotometer. The changes in yield of xylose was estimated by differentiating treatment time in autoclaved, having a fixed treatment temperature of 121°C, against a fixed loading of dilute acid having three different concentration of 1%, 3% & 5%, of 10% against each dried biomass taken.

The available pre-treatment procedure recommends dry heat, moist heat, chemical, physical and physicochemical methods. Upon then the auto hydrolysis procedure has shown a definite less use of energy & time. Also due to much available calorie on a particular volume than use of other pre-treatment methods shows a promising sustainability on industrial scale. Detailed studies were carried out with *Parthenium hysterophorus* to establish the variation of xylose yield.

II. MATERIALS AND METHODS

A. Preparation of substrate

After a peer review of all possible available substrate and their sustainability on all aspect like effect on nature, promising future, cost effectively, it is found that Parthenium may be the best possible choice of substrate. The easy available plants were collected from local areas surrounding CSIR-CMERI research laboratory were abundant for this work. The fresh green plant with almost healthy condition were brought to laboratory with enough care and cleaned with water vigorously to remove earthly impurities. The green plants were chopped into pieces and dried in a hot air oven at 106°C for 6 hours. The final dried mass is then grounded to a maximum size ranging 3-5 mm approx., before they stored for the rest of test.

B. Preparation of hemicellulose acid hydrolysate

1 g of dried Parthenium was mixed with 1%, 3% & 5% concentration sulphuric acid respectively to a final volume of 10 ml. The acid hydrolysis reactions were carried out in the temperature of 121°C, at 15 lb. pressure for a treatment time of 1 min, 2min, 3min, 4 min, 5 min 6 min, 7 min, 8 min, 9 min, 10 min, 15 min, 20 min, 25 min and 30 min, after which the hydrolysate was cooled down to room temperature. The hydrolysate was filtered using Wattman paper no.1 to remove the unhydrolysed material and solid residue. The filtrate was collected and subjected to analyze the xylose content.

C. Estimation of available xylose content in the biomass

Xylose, the majorly available pentose sugar in biomass reacts with Phloroglucinol in acidic medium to produce a purple blue, highly photosensitive Phloroglucinol-xylose complex. The amount of available xylose can be estimated by plotting the differentiation of concentration obtained from spectroscopic analysis report at 554 nm [7-8], against a known standard concentration analysis report.

D. Chemicals & reagents

Estimation of xylose by Phloroglucinol assay involves following chemical preparation before performing the test:

DI. D-xylose preparation

0.05 g of D-xylose was mixed with 10 mL of benzoic acid to prepare xylose solution (10 g/L).

DII. Phloroglucinol solution

0.5 gm. of Phloroglucinol was mixed with 100 ml of acetic acid to make 100 ml phloroglucinol solution.

DI. Benzoic acid solution

0.2 g of benzoic acid is mixed in 100 ml of distilled water, which was preheated at 60° C, and mixed well, then cooled and filtered to store the solution.

DI. ZnSO₄ solution

10 gm. of ZnSO₄ was added with 100 ml of distilled water

DII. NaOH solution

2 gm. of NaOH pellets was measured and added with 100 ml of distilled water.

DIII. Measuring of Xylose

To measure xylose 200 µl of sample was mixed with 5 ml of phloroglucinol solution and heated at 90°C for 5 min and cooled to room temperature. The samples were then measured with spectrophotometer and the absorbance was recorded at 554 nm [9].

DIV. Preparation of blank

200 µl of benzoic acid was added to 300 µl of ZnSO₄, 300 µl NaOH, 500 µl of concentrated HCl, then added 5 ml of phloroglucinol solution. The samples were heated at 90°C for 5 min and cooled to room temperature.

DV. Preparation of xylose standard

From previously made D-xylose solution, 0.4 g/L, 0.8 g/L, 1.2 g/L, 1.6 g/L, and 2.0 g/L concentration D-xylose was prepared for the standard curve measured at 554 nm in spectrophotometer. The average movements of thermo chemical reaction rate constant k depict the reaction state is of 1st order & it is significantly following Arrhenius law in the form of $k=Ae^{(-E/RT)}$, where A is the pre exponential factor and depends on the concentration.

III. RESULT AND DISCUSSION

Successful utilization of Parthenium in emerging green-fuel technology is limited. The purpose of this ongoing research is to emphasize on the kinetics of xylose conversion from hemicelluloses derived from *P. hysterophorus* L. the bio-chemo conversion of hemicellulose to xylose was successfully achieved by moist heat acid hydrolysis at approx. 15 lb. pressure. Auto hydrolysis of Parthenium by dilute acid yields mixture of sugars with xylose as a major component.

The effect of varying treatment time in autoclave, against a fixed treatment temperature and concentration of sulphuric acid on xylose yield from hemicelluloses derived from *P. hysterophorus* L. have been carefully investigated and represented in Fig 4.

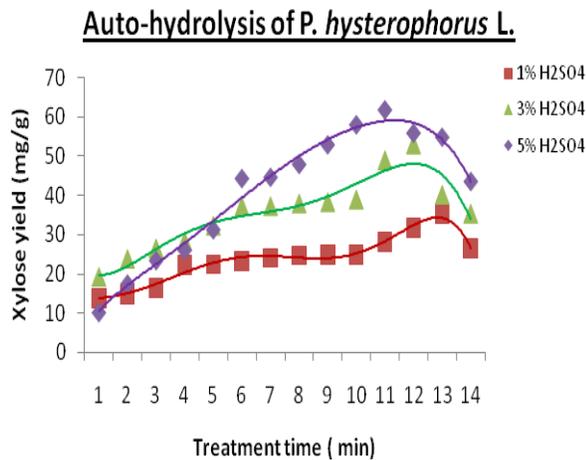


Fig 4: Xylose yield with change in treatment time and concentration of sulphuric acid at 121°C and 15 lb. pressure.

It has been noticed that an increase in acid concentration from 1% to 5% leads to increase in the xylose yield at a particular treatment condition. The maximum xylose yield from dried Parthenium was found up to 61.7 mg/g, when the acid hydrolysate was autoclaved at 121°C for 15 minute with 15 lb. pressure. The generation of furfural [10], a by-product of xylose degradation as a consequence of acid hydrolysis is kept on mind during the pre-treatment process. The rate of degradation depends on temperature and concentration of sulphuric acid [11]. The above result depict gradual increase in yield of xylose up to 15 minute treatment time with 1% & 3% acid and at a peak in 25 minute with 1% acid is there and then gradual degradation in xylose concentration is observed.

The reaction rate constant k is evaluated using integrated rate law. The rate constant value for each acid concentration is presented graphically in Fig 5.

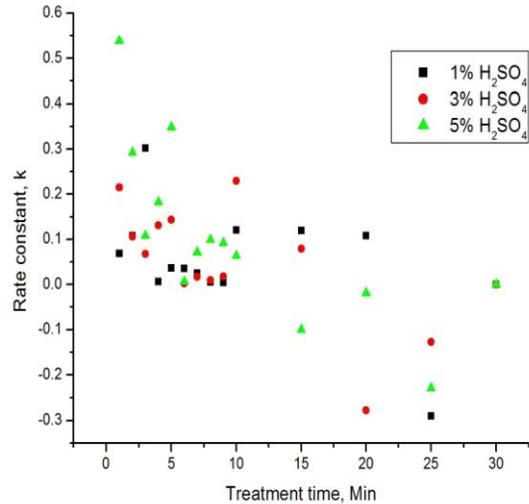


Fig 5: Variation of reaction rate constant with time.

It has been observed in Fig 6 that the rate constant increases steadily with the increase in acid concentration after a value of 3%. The initial decrease up to 3% acid concentration may be the effect of other parameters, e.g., particle size, particle loading, etc., which were not considered during this research work.

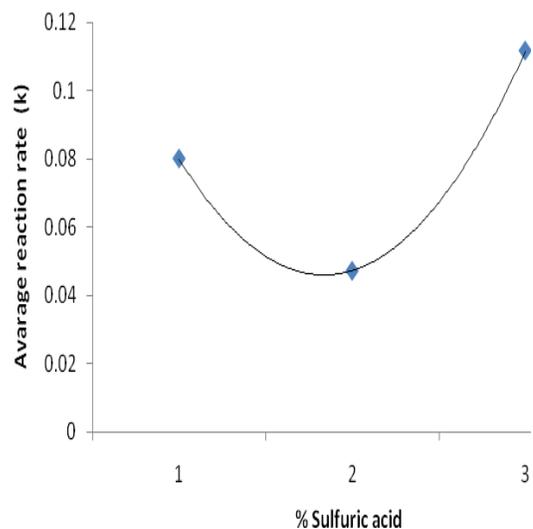


Fig 6: Effect of acid concentration on the rate constant.

IV. CONCLUSION

A number of biomass is already under extensive research for green fuel biotechnology. Here the newly addition of introduction of high growth eligible, obnoxious weed *Parthenium hysterophorus* will definitely create a promising arena as it was rarely used previously in such purposes [12]. Hydrolysis carried out with 1%, 3%, and 5% H₂SO₄ solutions in 121°C at 15 lb. pressure with treatment time of 1 min to 30 mins. From experimental results it has been found that xylose yield increases with increase in the treatment temperature of the biomass inside the autoclave & acid concentration. It is observed that under pressure the moist heat direct significant conversion of xylose rapidly. The maximum value of xylose yield obtained is around 61.7 mg/g. of dry *Parthenium*, when hydrolysed with 5% H₂SO₄ at a temperature of 121°C with 15 minute treatment time. The present work will encourage the researchers to explore the potential of one of the most obnoxious weed, *Parthenium hysterophorus* L for the generation of green fuel ethanol. Regression of kinetic data may be taken up as future work for optimization of parameters affecting the production of xylose from *parthenium hysterophorus*.

References

- [1] Exchange, N.Y.M., <http://www.nymex.com/>.
- [2] European Commission, World energy, technology, and climate policy outlook (WETO 2030), EUR 20366, 2003.
- [3] Y. Sun and J. Cheng, Hydrolysis of lignocellulosic materials for ethanol production: a review, *Bio resource Technology* 83, 2002, 1-11.
- [4] T. J. Ebert's, R. H. Sample, M. R. Glick and G. H. Ellis, A simplified colorimetric micromethod for xylose in serum or urine, with phloroglucinol, *Clin Chem.* 25, 1979, 1440-1443.
- [5] Dr. M. Mahadevappa: *Parthenium – A Dreaded Weed* International *Parthenium* research News, Vol 1, No 1, June 1, 2008.
- [6] H Lata, V.K. Garg, R.K.Gupta. Removal of a basic dye from aqueous solution by adsorption using *Partheniumhysterophorus*: an agricultural waste. *Dyes Pigment.* 2007;74:653–658. doi: 10.1016/j.dyepig.2006.04.007.
- [7] T. J. Eberts, R. H. Sample, M. R. Glick and G. H. Ellis, A simplified colorimetric micro method for xylose in serum or urine, with phloroglucinol, *Clin Chem.* 25, 1979, 1440-1443.
- [8] S. L. Johnson Bliss M, Mayersohn and K. A. Conrad, Phloroglucinol-based colorimetry of xylose in plasma and urine compared with a specific gas-chromatographic procedure, *Clin Chem.* 30, 1984, 1571-1574.
- [9] C. Pronyk and G. Mazza, Kinetic Modeling of Hemicellulose Hydrolysis from Triticale Straw in a Pressurized Low Polarity Water Flow-Through Reactor, *Ind Eng Chem.* 49, 2010, 6367 – 6375.
- [10] M. Ackerson, M. Ziobro and J. L. Gaddy, Two-stage acid hydrolysis of biomass, *Biotechnol Bioeng Symp*, 11, 1981, 103-112.
- [11] G. Gonzales, J. Lopes-Santin, G. Caminal and C. Sola, Dilute acid hydrolysis of wheat straw hemicellulose at moderate temperature: a simplified kinetic model, *Biotechnol Bioeng*, 28, 1986, 288-293.
- [12] H.P. Singh, D.R. Batish, J.K. Pandher, R.K. Kohli . Assessment of allelopathic properties of *Parthenium hysterophorus* residues. *Agric. Ecosy. Environ.* 2003, 95: 537-541.