Microbial xylanases for paper industry

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An overview presentation is made on the potential application and current global status on the use of xylanase enzymes in paper and pulp biotechnology. Emphasis is on the need to screen and identify suitable xylanolytic enzymes compatible with availability of indigenous raw materials for pulp manufacture; i.e. importance of cellulase-free alkali-stable xylanase enzymes, particularly for developing viable technologies to effect pulp bleaching. Substantial reduction in the use of toxic chlorine compounds in the paper industry, which are currently in use for achieving increased pulp brightness, can be envisaged.

THE past few decades of the twentieth century have witnessed spectacular advances and betterment of living standards due to the beneficial integration of novel and brilliant ideas with scientific progress and rapid translation of laboratory findings into practical technologies and commercial-scale manufacturing processes. In the field of chemical technology, where manufacture of a variety of products on large scale has resulted in serious effluent and hazardous waste disposal problems, the need for safer and 'environmental friendly' technologies has become imminent. This has resulted in scientists attempting to learn from natural processes, and therefore a new aspect of microbiology and biotechnology has rapidly begun to gain ground. Microorganisms perform their myriads of biochemical reactions under ambient conditions with little or no toxic and hazardous by-products. Therefore enzyme alternatives to polluting chemical technologies, which began initially as an idea, have reached realistic proportions whereby they can be considered as worthwhile and practical technologies of the future. A glimmer of hope to save the environment and yet achieve the goals of chemical technology is gaining momentum in several frontier developments in this area. One such area is the pulp and paper industry where the quantities of raw materials processed are huge, as well as the use of naturally hazardous chemicals are also large. Progress through intense research and development activities to make the technology eventually totally free from hazardous chemicals would be a cherished dream come true. In the present article, an overview is presented on the present status and future prospects on the use of xylanase enzymes as effective bioreagents to achieve biobleaching in place of poisonous chlorine compounds conventionally used to achieve pulp brightness of a high order in the manufacture of high-quality paper products.

Chemical structure and distribution of xylan in plant biomass

Xylan is the most abundant noncellulosic polysaccharide present in both hardwoods and annual plants, and accounts for ≃ 20–35% of the total dry weight in tropical plant biomass. In temperate softwoods, xylans are less abundant and may comprise about 8% of the total dry weight. Xylan is found mainly in the secondary cell wall and is considered to be forming an interphase between lignin and other polysaccharides. It is likely that xylan molecules covalently link with lignin phenolic residues, and also interact with polysaccharides, such as pectin and glucan. In simplest forms, xylans are linear homopolymers that contain D-xylose monomers linked through *B*-1,4-glycosyl bonds (Figure 1). In nature, they are partially substituted by acetyl 4-*O*-methyl-D-glucuronosyl and L-arabinofuranosyl residues, forming complex heterogenous and polydispersed polymers. Many structural aspects of xylans are unclear because of the difficulties associated with the isolation of xylans from natural raw materials without significant alteration or loss of the original structure and association with other components.

Diversity of structural features associated with the xylan component from different hard woods and soft woods makes it obvious that there is an undisputed necessity for different xylan-degrading enzymes suited to different lignocellulosic substrates used as source materials for the paper industry in different parts of the world. In other words, xylanase technology which is optimized for the soft-wood-based paper industry in the western world will not directly be applicable to the hard-wood-based paper industry of India and other developing countries of the tropical regions.

Effect of pulping operations on xylan

Viikari *et al.*[1] while discussing the important properties of the xylanases for use in the pulp and paper industry, have also discussed in detail the effect of pulping operations on the hemicellulose component of the plant biomass subjected to kraft pulping. Extensive modifications in the

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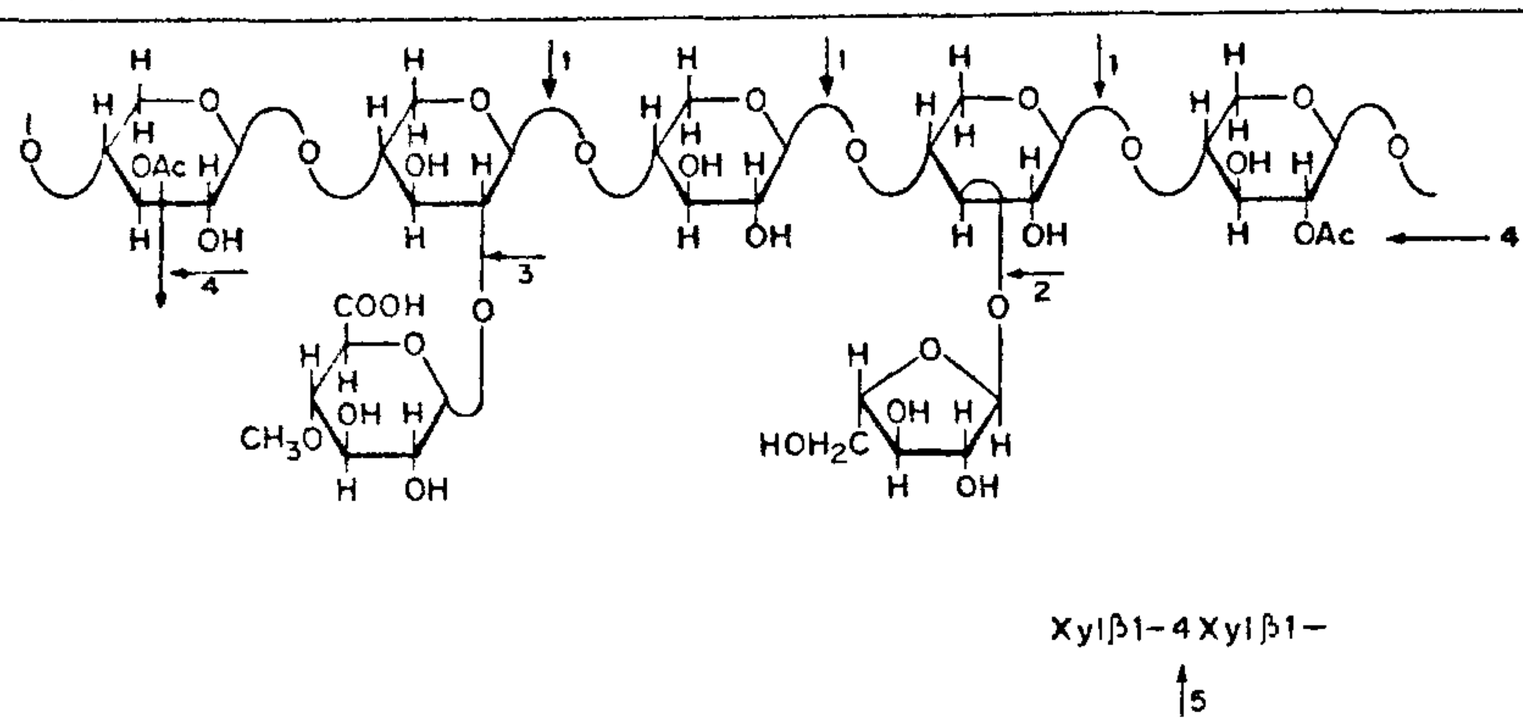

Figure 1. Structure of xylan and enzyme cleavage sites.

chemical structure, including removal of the acetyl groups and decrease in the glucuronic acid side groups, are observed. With the high-alkali concentration during the kraft cook, part of the xylan is dissolved in the pulping liquor; while short-chain xylan precipitates in a more or less crystalline form on the surface of cellulose microfibrils. This xylan forms a barrier against effective extraction by chemicals of the residual brown-coloured lignin from the fibres. As a result, quantities of chlorine or chlorine-containing compounds are required to be used for effective reduction in the kappa number, and increase in pulp brightness. Enzymatic solubilization of the hemicellulose settled on the pulp fibres would be an 'environmentally compatible' technology to improve the accessibility of the brown lignin to chemical bleaching together with substantially reduced quantities of bleaching chemicals required to achieve the same degree of bleaching and brightness.

Microbial sources of cellulase-free xylanase production

Microorganisms are rich sources of xylanase enzymes, which are produced by diverse genera and species of bacteria, actinomycetes and fungi. While several *Bacillus* species secrete high levels of extracellular xylanase, filamentous fungi secreting high amounts of extracellular proteins, xylanase secretion often accompanies cellulolytic enzymes – for example as in species of *Trichoderma*, *Penicillium*, and *Aspergillus*. To use xylanase enzymes for pulp treatment, it is preferable not to have any accompanying cellulolytic activity, since the cellulase may adversely affect the quality of the paper pulp. The topic of microbial xylanase production has been extensively reviewed by several groups[2–9].

Some of the initial approaches for overcoming cellulase activity in xylanase preparations included treatment with mercurial compounds to selectively inhibit cellulase, or cloning and selective expression of xylanase genes in heterologous host systems. But perhaps the most practical approach has been in the screening for naturally occurring microbial strains that are capable of secreting cellulase-free xylanases under optimized fermentation conditions. A summary of such organisms and their enzyme characteristics has been presented by Srinivasan and Rele[10]. Besides overcoming cellulase activity and conferring stability to xylanases at high temperatures (usually 60–70°C, which is also at the temperature of the incoming pulp for the bleaching operation), the highly alkaline conditions prevailing in the pulp would also require that xylanases remain active and stable under the high alkaline pH conditions.

Among the early reports of successful identification of cellulase-free xylanases, our studies on *Chainia* sp. NCL 82-5-1 isolated from the desert sands of Rajasthan deserves a mention. While this sclerotial actinomycete strain secreted 8–10 IU/ml of endoxylanase on media formulated with commercial ingredients rich in xylan such as cereal brans, up to 26 IU/ml was secreted on pure xylan media in submerged cultures. The xylanase was active optimally at pH 5–7 and 55–60°C, and the crude culture filtrates did not show any detectable cellulase activity towards either carboxymethylcellulose or filter paper[11–12]. Cellulase-free xylanase activity has been reported by several workers and these include the xylanase enzymes from *Streptomyces roseiscleroticus*[13], *Saccharomonospora viridis*[14], and

Table 1. Properties of xylanases produced by alkalophilic microbes

Source	Molecular mass (kDa)	pI	Optimum pH (reaction temp.°C)*	Optimum temp.°C (reaction pH)**	Reference
Bacteria					
Aeromonas sp. 212	145	–	7.0–8.0 (40)	50	43
	37	–	6.0–8.0 (40)	50	
	23	–	5.0–7.0 (40)	60	
Bacillus xylanases					
Bacillus sp. C-59-2	–	6.3	5.5–9.0 (40)	60 (7.0)	18
Bacillus sp. C-125	43	–	6.0–10.0 (40)	70 (7.0)	44
	16	–	6.0–7.0 (40)	70 (6.0)	
Bacillus sp. NCL 87-6-10	10.4	–	8.0–9.0 (50)	60 (8.0)	19, 20
Bacillus sp. YC-335	40	–	6.0 (40)	55	45
Bacillus sp. NG-27	–	–	7.0,8.4 (65)	70	24
Bacillus Circulans	–	–	7.0–8.0	45	46
Bacillus sp. (VI-4)	–	9.1	6.0–7.0 (55)	55 (7.0)	47
Bacillus sp. Sam III	–	–	8.0 (60)	60 (8.0)	48
Bacillus sp. 41M	3.6	5.5	9.0 (37)	50 (9.0)	23
Bacillus sp. W1	21.5	8.5	6.0 (60)	65 (6.0)	49, 50
	49.5	3.6	7.0–9.0 (60)	70 (6.0)	
Bacillus sp. W2	22.5	8.3	6.0 (60)	65 (6.0)	49, 50
	50	3.7	7.0–9.5 (60)	70 (6.0)	
Bacillus sp. W3	–	–	6.0 (60)	65 (6.0)	49, 50
Bacillus sp. W4	–	–	6.0–7.0 (60)	70 (6.0)	
Bacillus sp. 91	–	–	7.5 (60)	65 (7.5)	51
Bacillus thermoalkalophilus	–	–	6.0–7.0 (60)	60–70 (7.0)	21
Bacillus sp. NCIM 59	35	4.0	6.0–8.0 (50)	50–60 (7.0)	
	15.8	8.0	6.0–8.0 (50)	50 (7.0)	22
Bacillus sp. TAR-1	40	4.1	5.0–9.5 (50)	75 (7.0), 70 (9.0)	52
Bacillus stearothermophilus	43	9.0	6.0–7.0 (55)	65–70 (7.0)	25
Actinomycetes					
Nocardiopsis dassonvillei	23	4.9	7.0 (50)	60 (7.0)	53
	23	5.3	7.0 (50)	60 (7.0)	53
	37	4.1	7.0 (50)	50 (7.0)	53
Streptomyces sp. VP-5	–	–	4.8–10.0 (50)	50	26
S. Viridosporus T7A	59	10.2	7.0–8.0 (60)	65–70 (7.0)	54
Fungi					
Cephalosporium sp. (NCL 87-11-9)	28	4.05	6.5–9.0 (40)	40 (7.0–9.0)	27, 28
Cephalosporium RYM-202:					
Enzyme CX-I	35	6.3	7.5 (50)	50 (7.5)	
Enzyme CX-II	24	4.4	8.0 (50)	50 (8.0)	55
Aspergillus fischeri Fxn-1	–	–	6.0–6.5	60 (6.0)	29

*Reaction temperature °C in this column is enclosed in parenthesis.
**Reaction pH in this column is enclosed in parenthesis.

thermolotolerant *Streptomyces* sp. T-7 (refs 15, 16); all these enzymes are active at or around neutral pH values.

In keeping with the requirements of the pulping operations, the search for cellulase-free xylanases from diverse microbial strains that are active under high alkaline pH conditions having different temperature stabilities have gained momentum, and several reports of alkalophilic microorganisms producing active xylanases with the desired properties have been published (Table 1). Srinivasan and Rele[17] published a comprehensive review on microbial cellulase-free xylanases and their applications in pulp and paper biotechnology. Horikoshi and Atsuka[18] were the first to report a xylanase from *Bacillus* sp. that was active under high pH conditions. An obligately alkaline *Bacillus* sp. strain isolated from rotting coconut fibres by Srinivasan *et al.*[19] secreted high levels of xylanase in commercial media containing wheat bran and organic nitrogen, and the enzyme while being optimally active at 60°C at pH 8.0, retained over 75% of its activity at pH 9.0 (ref. 20). Several promising *Bacillus* strains, including alkalo-thermotolerant ones, have been investigated and their xylanase production as well as

enzyme characterization published. While Rajaram and Varma[21] isolated from termite-infested soil mounds *B. thermoalkalophilus* that resulted in high yields of xylanase using cheap agricultural wastes, such as rice husk and bagasse, as its substrates. Dey *et al.*[22] characterized from an alkalothermophilic (AT) *Bacillus* strain, a xylanase comprising two components active over a pH range of 6–10 and temperature range of at 50–60°C. An alkalotolerant *B. circulans* was described by Nakamura *et al.*[23], producing high activity xylanase at pH 8–8.5 on a medium containing beechwood xylan. Other reports of thermostable xylanases from various species of *Bacillus* include those of Gupta *et al.*[24] and Khasin *et al.*[25]. Vyas *et al.*[26] reported an alkalophilic *Streptomyces* sp. secreting cellulase-free xylanase that was active above pH 9.0. While the first report of cellulase-free xylanase secretion from an alkalophilic fungus, *Cephalosporium* sp. NCL 87-11-9 was published by Bansod *et al.*[27,28], Chandra Raj and Chandra[29] described a cellulase-free xylanase from *Aspergillus fischeri* that was alkali tolerant and stable in the pH range of 5–9.5, and retained its activity at 60°C.

Technological application of xylanases in pulp and paper industry

Application of biotechnology to pulp and paper industry operations is no longer an academic or potentially useful alternative proposition for the future. In the developed world, several commercial xylanases with varying activities as well as pH and temperature stabilities have been applied on semi-commercial or commercial scale to pulping operations and extensive data have been collected and discussed in several symposia organized between 1992 and 1996. Several authoritative review articles have been published which provide information and data on the efficacy of xylanase enzymes in pulp biotechnology[30–33]. In his review on the enzymatic treatment of pulps, Jeffries[34] emphasized the diversity observed in pulp operations and therefore there exists a vast potential for variability in the choice of enzymatic treatments as well as in the choice of various microbial enzymes.

Enzyme application improves pulp fibrillation and water retention, reduction of beating times in virgin pulps, restoration of bonding and increased freeness in recycled fibres, and selective removal of xylan from dissolving pulps. Xylanases are also useful in dissolving pulps, yielding cellulose for rayon production, and biobleaching of wood pulps[35]. Application of xylanases together with other bleach agents, such as oxygen and hydrogen peroxide in pulp industry has been extensively investigated and projections of a totally chlorine-free pulp technology have been put forward[36]. Pulpzyme HA, introduced by Novo Nordisk A/S, was the first commercially available xylanase for use in biobleaching of wood pulps. It was extracted from a strain of *Trichoderma reesei* and was used in the first bleaching stage to reduce the dosage of active chlorine. Several multinational biotech companies are marketing various xylanase preparations, such as Irgazyme (Genencor International), Cartazyme (Sandoz), Ecopulp (Alko), and VAI xylanase (Voest Alpine). Enzymatic pre-bleaching has been successfully demonstrated on mill scale wherein a pulp with 88% ISO brightness was achieved when used together with chlorine dioxide and hydrogen peroxide[37]. The following data published from Finland of a large-scale mill trial would enable a better appreciation of enzymatic pre-bleaching with xylanase: 35 tons of Albazyme 10 was used to treat and produce 35,000 tons of fully bleached pulp from hard wood as well as soft wood. The enzyme was added to a kraft-cooked pulp, after suitably adjusting the pH and temperature to suit the optimum conditions for enzyme activity. An overall reduction of 12% chlorine use

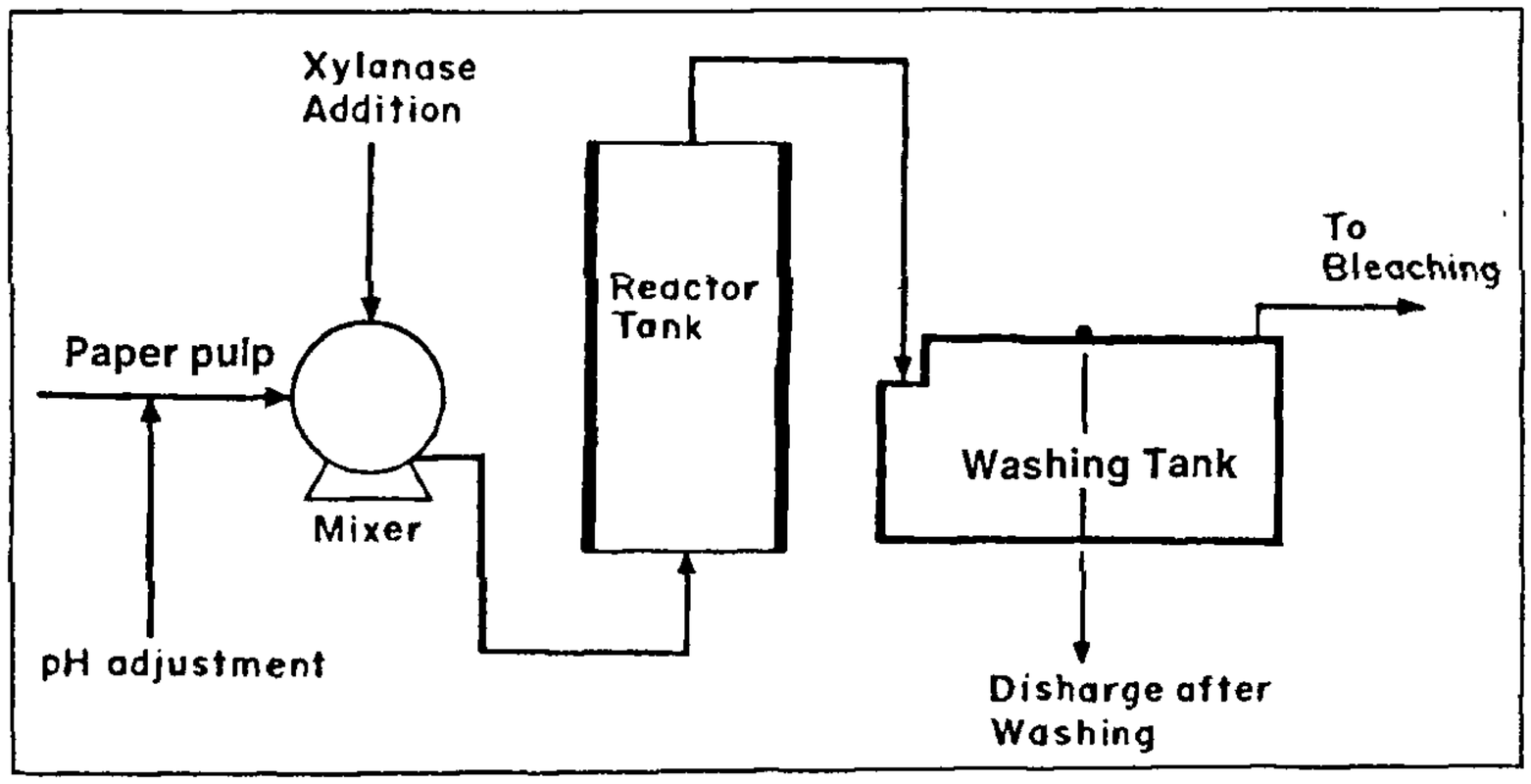

Figure 2. Xylanase application process flow-sheet.

could be achieved. Lundgren *et al.*[38] have reported mill trials on soft-wood pulp using a xylanase optimally active at pH 9.0 and 65°C. They observed that in the pulping sequence where chlorine was totally eliminated, the pulp bleaching and brightness were satisfactory and also consumed lesser quantities of H_2O_2. This report is an example of applying an alkali-stable and heat-stable xylanase for large-scale pulp biotechnology.

Current efforts are aimed at process optimization, simplification, and cost reduction of enzyme application in pulp industry. Nisson *et al.*[39] have pointed out that with the xylanases available commercially at present, a pH adjustment of the incoming pulp from pH 10–11 to 6–8 is necessary for its optimal activity. From an industrial point of view, it is simple to adjust the pH but difficult and expensive to control temperature due to the cost of cooling. The ideal solution therefore would be to use enzymes with higher pH and temperature stability, which will make the large-scale operations more simple and cost effective. It is thus obvious that the focus of future developments will be on identifying xylanases with higher thermostability at high alkaline pH, and developing process technologies for commercial-scale manufacture of such enzymes. In this context, alkalophilic and alkalo-thermophilic *Bacillus* strains as well as xylanolytic thermophilic bacteria, viz. *Dictyoglomus* sp.[40–42] may possess the right combination of gene pools which could be gainfully employed in future for developing the ideal strains suited for pulp biotechnology. A diagrammatic flowchart involving xylanase in pulping technology is represented in Figure 2.

Besides xylanases, mannanases as well as side-chain-cleaving enzymes, such as alpha-arabinofuranosidase, have also been evaluated depending upon individual raw materials used under different situations for paper manufacture.

Pulp and paper biotechnology in the Indian context: an appraisal

In the global context of switch over to biotechnology, the other alternative being used for pulp bleaching using chlorine is viewed with positive disfavour. Therefore, it becomes essential that paper industry in India too should opt for the 'enzyme alternative' at the earliest. The day may not be far off when paper products manufactured with chlorine compound-based technology are prohibited for wrapping food products and other consumer items so that our export markets do not suffer.

If we have to develop indigenous enzyme technology to suit the indigenously available raw materials for paper manufacture, we have to evolve strategies that generate viable technologies for xylanases' production, based on original discoveries. Programmes involving microbiologists, biochemists, process engineers have to be coordinated; and in collaboration with the paper industry the problem must be looked at in total perspective to create effective and functional networks which are progress- and result-oriented. Innovative approaches for the screening of novel xylanolytic microbial strains from mesophilic as well as extreme environments must be undertaken with full vigor to explore, isolate, and conserve in germplasm banks a diversity of microbial species on which basic microbiological studies as well as recombinant DNA explorations can be undertaken for obtaining xylanase enzymes with novel properties that can be eventually exploited commercially. Realistic cost estimates and improvement in process economics are the key factors in the commercial success of any technology and therefore it must be clearly understood that no enzyme-based process for bleaching can be as inexpensive as using chlorine or even organic chlorine compounds. Thus, the added expenses incurred by the use of enzymes must be viewed in terms of their accrued indirect benefits like prevention of environmental derangement and reduced health hazards to mankind.

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