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(21) International Application Number: PCT/DK93/00217 (22) International Filing Date: 2 July 1993 (02.07.93) (30) Priority data: 0869/92 2 July 1992 (02.07.92) DK 1030/92 20 August 1992 (20.08.92) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bags- værd (DK). (72) Inventor; and (75) Inventor/Applicant (for US only) : YDE, Birgitte [DK/DK]; Tornehøj 148, DK-3520 Farum (DK). (74) Common Representative: NOVO NORDISK A/S; Patent Department, PeV, Novo Allé, DK-2880 Bagsværd (DK).		(81) Designated States: BR, CA, FI, NO, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: POLYMERIZATION OF LIGNIN AT ALKALINE pH		
(57) Abstract <p>The present invention relates to a method of enzymatic polymerization and/or modification of lignin or lignin containing materials. The invention also relates to the use of this method in the manufacture of wood composites or wood laminates.</p>		

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Polymerization of lignin at alkaline pH.

TECHNICAL FIELD

The present invention relates to a method of enzymatic polymerization and/or modification of lignin or lignin containing materials. The invention also relates to the use of this method in the manufacture of wood composites or wood laminates.

BACKGROUND ART

Wood composites such as fibre board and particle board are made by binding the material (e.g. wood fibres or wood particles) with a suitable binder and pressing, usually with heating. Conventional chemical binders for this purpose are e.g. urea-formaldehyde resin and phenol-formaldehyde resin.

Binders made from enzymatically treated lignin are also known. Thus, it has been suggested to use a binder made from peroxidase treated lignin (vide e.g. US Patent No. 4,432,921), but the use of a peroxidase has not previously been demonstrated.

For wood composites, e.g. for use in construction, a high physical strength is generally important. In order to obtain high physical strength it is essential that an optimal polymerization/modification takes place during binding of the material.

As a liquid binder material is believed to be a prerequisite for optimal polymerization, enzymatic polymerization processes, due to the reaction pH of the enzymes hitherto employed, optionally involve the addition of organic solvents in order to solubilize lignin (vide e.g. EP Patent Application No. 354,485).

Another way of improving the strength of the composite board involves addition of chemical polymerization agents, e.g. polydiphenylmethyldiisocyanate (PMDI), (vide e.g. EP Patent Specification No. 275,544).

SUMMARY OF THE INVENTION

It is the object of this invention to provide a method of polymerization of lignin or lignin containing materials that allows for optimal polymerization/modification at elevated pH without need for organic solvents to be added.

We have now surprisingly found that peroxidases are able to polymerize lignin or lignin containing material at elevated pH values, by which there is no need for organic solvents to be added to the reaction mixture, provided the polymerization is conducted by a controlled addition of hydrogen peroxide during the reaction.

The invention provides a method of enzymatic polymerization/modification of lignin or lignin containing materials, the method being improved in respect of polymerization and exploitation of the lignin. Moreover, the method does not require the addition of organic solvents or chemical polymerization agents.

Due to the extent of the polymerization achieved by this method, it is especially advantageous for implementation in the manufacture of wood composites or laminated wood products of which high physical strength is required.

Accordingly, the invention provides a method of enzymatic polymerization and/or modification of lignin or lignin containing material by treatment of the lignin or lignin containing material with an aqueous solution of peroxidase at elevated pH values, and under a controlled addition of hydrogen peroxide, in the absence of organic solvents.

In another aspect, the invention provides a glue or active binder obtained by subjecting lignin sulphonate to the method of the invention.

In yet another aspect, the invention relates to the use of the method of the invention in the preparation of a glue or an active binder for use in the manufacture of wood composites e.g. wood fibre materials such as chipboards, fibre boards, or particle boards, or in the manufacture of laminated wood products, such as laminated beams and plywood.

BRIEF DESCRIPTION OF DRAWINGS

The present invention is further illustrated by reference to the accompanying drawings, in which:

Figs. 1A-1D shows polymerization of Indulin™, determined by average molecular weight (MW x 1000), by use of SP 502™ (4000 PODU/ml) and 10 mM H₂O₂. (A Start; B 5 min; C 30 min; D 60 min; E 65 min; F 90 min);

Fig. 1A Standard: 4000 PODU/ml and 10 mM H₂O₂;

Fig. 1B Extra Enzyme: 2 x 4000 PODU/ml;

10 Fig. 1C Extra peroxide: 2 x 10 mM H₂O₂;

Fig. 1D 2 x extra peroxide: 3 x 10 mM H₂O₂;

Figs. 2A-2D shows optimization of enzyme dosage for polymerization of Indulin™, determined by average molecular weight (MW x 1000), by use of SP 502™ and 10 mM H₂O₂ (A Start; 15 B 5 min; C 2 x 10 mM H₂O₂);

Fig. 2A: 10 PODU/ml;

Fig. 2B: 40 PODU/ml;

Fig. 2C: 400 PODU/ml;

Fig. 2D: 4000 PODU/ml;

20 Figs. 3A-3D shows polymerization of Indulin™ at various temperatures, determined by average molecular weight (MW x 1000), by use of SP 502™ and 3 x 10 mM H₂O₂ (A 400 PODU/ml, 20 °C; B 400 PODU/ml, 40 °C; C 4000 PODU/ml, 20 °C; D 4000 PODU/ml, 40 °C);

25 Fig. 3A: Start;

Fig. 3B: 5 min;

Fig. 3C: 10 min;

Fig. 3D: 15 min;

Figs. 4A-4D shows polymerization of Indulin™ at 30 various pH, determined by average molecular weight (MW x 1000), by use of SP 502™ (400 PODU/ml) and 3 x 10 mM H₂O₂ (A pH 7.2; B pH 8.6; C pH 9.5);

Fig. 4A: Start;

Fig. 4B: 5 min;

35 Fig. 4C: 10 min;

Fig. 4D: 15 min; and

Figs. 5A-5F shows polymerization of various lignins determined by average molecular weight after treatment for 30 minutes with SP 502™ (4000 PODU/ml) (A Blank test; B Sample);

Fig. 5A: Indulin;

5 Fig. 5B: Lignin 6;

Fig. 5C: Lignin S;

Fig. 5D: Fichtenlignin 4;

Fig. 5E: Fichtenlignin 6;

Fig. 5F: Fichtenlignin 9.

10

DETAILED DISCLOSURE OF THE INVENTION

The present invention provides a method of enzymatic polymerization of lignin or lignin containing materials, which method comprises the action of a peroxidase at elevated pH values and a controlled addition of hydrogen peroxide. The
15 method provides an active binder that may be used in the manufacture of wood composites, or as glue in the manufacture of laminated wood products.

The Enzyme

According to the invention, it has now been found
20 that peroxidases (EC 1.11.1.7), preferably the peroxidases derived from Coprinus, e.g. C. cinerius or C. macrorhizus, or Bacillus, e.g. B. pumilus, are superior to the enzymes hitherto used in enzymatic polymerization processes.

Surprisingly, and contrary to the enzymes hitherto
25 used in enzymatic polymerization methods, it has turned out that peroxidases possess excellent polymerization even at highly alkaline pH values. At alkaline conditions lignin becomes water soluble, and, thus, there is no need to add organic solvents. The presence of a liquid binder material allows for maximal
30 polymerization, leading to products of improved physical resistance. Moreover, a liquid binder, or at least a colloidal binder, is more easily dispensed through valves, nozzles or jets, usually employed in the manufacture of wood composites or laminated wood products.

The method shall be performed at alkaline conditions, i.e. pH 7-13. In particular alkaline conditions in which the enzyme possesses sufficient polymerization activity, i.e. pH 8-12 is preferred. In its most preferred embodiment, the method is performed at pH 9-11.

The amount of peroxidase should generally be in the range of 10-10,000 PODU per g of lignin or lignin containing material (PODU is a unit of peroxidase activity defined below).

Addition of Hydrogen Peroxide

10 The presence of hydrogen peroxide is essential to the action of peroxidases. A suitable amount of H_2O_2 will usually be in the range of 0.01-20 mM, preferably 1-10 mM.

However, in order to secure maximum performance of the enzyme and to avoid unintended degradation of the highly 15 reactive and costly hydrogen peroxide, the addition of hydrogen peroxide should be conducted carefully throughout the incubation period.

Hydrogen peroxide may be added in separate portions (discretely), either primary in the initial stage of the 20 incubation period, or equally throughout the incubation period. Hydrogen peroxide may also be added continuously, either primary in the initial stage of the incubation period, or equally throughout the incubation period.

The addition of hydrogen peroxide may be conducted 25 manually or automatically, e.g. by use of a pump.

In another embodiment, addition of hydrogen peroxide may be conducted enzymatically by use of a hydrogen peroxide generating enzyme in the presence of its substrate, e.g. a glucose oxidase. The addition of hydrogen peroxide can also 30 take place using a chemical substance that liberates hydrogen peroxide.

Peracids may replace hydrogen peroxide in the process of the invention.

Lignin Containing Materials

The method of the invention finds application on lignin and/or lignin containing materials, e.g. lignocellulosic material. The lignin may be sulfonated or non-sulfonated.

5 Lignin or lignin containing materials are obtainable as byproducts from the pulping industry. Indulin, Lignin 6, Lignin S, Fichtenlignin 4, Fichtenlignin 6, Fichtenlignin 9, and Black Liquor are trade marks of lignin products, obtainable from various paper mills.

10 Incubation

The enzyme reaction emanates from an aqueous solution of the enzyme, into which the lignin or lignin containing material is immersed. The presence of water is essential to the enzymatic reaction. Furthermore, the amount of water should be
15 the amount sufficient to attain a suitable liquid or colloidal binder material, and it is typically in the range of 1-10 times the weight of lignin. At alkaline conditions, however, lignin becomes more easily solubilized, thereby making it possible to attain a liquid or at least a colloidal binder material with
20 less water.

Organic solvents, either water miscible or water immiscible, may be present during incubation, but are, however, not required. The method of this invention is carried out essentially without any organic solvents present, or in the
25 presence of minor amounts of organic solvents. In the context of this invention, essentially without any organic solvents present indicates less than 5% w/w, preferably less than 2%, most preferred less than 1% w/w of organic solvents present. In a more specific embodiment, the method is carried out in the
30 absence of organic solvents. Higher concentrations of organic solvents may hamper the enzyme performance, the reason why it is recommended to immobilize the enzyme.

Also the presence of chemical polymerizing agents, e.g. polydiphenylmethanediisocyanate (PMDI), is not a pre-
35 requisite of the present invention. The method of the invention may, therefore, be carried out in the presence of a chemical polymerizing agent.

The incubation may be performed at temperatures in the range of 20-90°C, preferably 20-70°C, and for a period of from 1 minute to several days, preferably 5 minutes to 5 hours, depending on the stability of the particular enzyme.

5 Peroxidase Activity

One peroxidase unit (PODU) is defined as the amount of enzyme which, under standard conditions (i.e. pH 7.0, temperature 30°C, and 3 minutes reaction time) catalyses the conversion of 1 μ mol hydrogen peroxide per minute. The activity
10 is determined using an assay based on ABTS® (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)) as the chromophore, the greenish-blue colour produced being photometered at 418 nm.

A folder AF 279/2 describing this analytical method is available upon request to Novo Nordisk A/S, Denmark, which
15 folder is hereby included by reference.

Wood Composite or Laminated Wood Products

The method of the invention may be used for the preparation of a glue or an active binder, which glue or active binder may be used per se in the manufacture of wood com-
20 posites, or in the manufacture of laminated wood products.

Alternatively, the method may be used as an integrated part of the manufacture of wood composites, or in the manufacture of laminated wood products, e.g. by spraying.

In particular, the method finds application in the
25 manufacture of wood composite or laminated wood commonly used in construction, where a high physical strength is generally important. Examples of wood composites are fibre board made from wood fibres, and particle boards (chipboards) made from wood particles. Examples of laminated wood products are plywood
30 or laminated beams.

A method of producing wood composites may include binding the material (e.g. wood fibres or wood particles) with the binder prepared by the method of the invention and pressing, usually with heating.

After the enzyme treatment water is drained off, e.g. by screening or squeezing, to obtain a "fleece" which is sufficiently solid for further handling.

The fleece is pressed at a temperature and time
5 sufficient to effect enzymatic polymerization of lignin in the fibre material and obtain a final product of the desired density and physical strength. The pressing may be cold (e.g. 40-70°C for 2-48 hours) or hot (e.g. 150-200°C for 2-20 minutes). A conventional press for production of wood com-
10 posites may be used.

The following examples further illustrate the present invention, and they are not intended to be in any way limiting to the scope of the invention as claimed.

EXAMPLE 1

15 The lignin used in this Example was Indulin™. The enzyme used was SP 502, a liquid peroxidase preparation having an activity of 10,000 PODU/g, available from Novo Nordisk A/S, Denmark. The enzyme preparation was diluted with buffer to an activity of 1000 PODU/ml. Samples were prepared the following
20 way.

Suspensions of 25 mg/ml of lignin in 0.4 M Britton & Robinson buffer were heated for 15 minutes at 80°C by shaking the samples on a water bath. The samples were centrifuged for 10 minutes at 2000 r.p.m., and the supernatant filtered,
25 thereby obtaining a lignin solution.

An enzyme blank was prepared by mixing 1200 µl of enzyme solution (1000 PODU/ml) with 1800 µl of Britton & Robinson buffer, followed by addition of H₂O₂ to an amount of 10 mM in the assay.

30 A blank was prepared by mixing 1800 µl of the above obtained lignin solution with 1200 of Britton & Robinson buffer, followed by addition of H₂O₂ to an amount of 10 mM in the assay.

Tests were prepared using 1200 µl of enzyme solution
35 (1000 PODU/ml) with 1800 µl of the above obtained lignin

solution, followed by addition of H₂O₂ to an amount of 10 mM in the assay.

The tests were performed at pH 7.2, pH 8.6, and pH 9.5, respectively. Samples were collected after 5 minutes, 10 minutes, and 15 minutes, respectively, and the tests were performed at room temperature (20°C) unless otherwise stated.

Average molecular weight determination was determined using HPLC, as a GPC-method.

The results of these optimization experiments are presented in the appended Figs. 1-5.

Fig. 1 shows that a "conventional" polymerization reaction is able to increase the average molecular weight (AMW) to appr. 40,000. If additional enzyme is added (after 5 minutes of incubation) the AMW is increased to appr. 50,000. If additional hydrogen peroxide is added (after 30 minutes of incubation) AMW increases to appr. 125,000, and if hydrogen peroxide is added twice (after 30 and 60 minutes, respectively) an AMW of as high as appr. 175,000 is achieved, which is appr. four times the AMW of a "normal" polymerization.

Fig. 2 shows optimization of enzyme dosage. It appears that the polymerization reaction increases with increased enzyme dosage, but optimum performance is within the range 10-40 PODU/ml. It also shows the effect of additional hydrogen peroxide, added after 5 minutes of incubation. AMW are two-three fold increased.

Fig. 3 shows polymerization at 20 and 40°C, respectively.

Fig. 4 shows polymerization at pH 7.2, 8.6, and 9.5, respectively. At pH 7.2, 8.6 and 9.5 the degree of polymerization after 15 minutes is higher for the reactions performed at pH 8.2 and 9.5 than at pH 7.2. At pH 7.2 the polymerization starts immediately, but at pH 8.2 and 9.5 the polymerization is relatively slow for the first 5 and 10 minutes, respectively, whereafter the reaction velocity is much faster than at pH 7.2.

Fig. 5 shows AMW after 30 minutes of polymerization of various lignins.

EXAMPLE 2

The lignin used in this Example was Black Liquor™. The enzyme used was SP 502, a liquid peroxidase preparation having an activity of 10,000 PODU/g, available from Novo Nordisk A/S, Denmark. The enzyme preparation was diluted with buffer to an activity of 1000 PODU/ml. Samples were prepared the following way.

For the first test a solution containing 14.0 mg/ml of lignin in a buffer (Milli-Q™), pH 10,8 was prepared.

10 For the second test a solution containing 14.2 mg/ml of lignin in a fosfate buffer, pH 8.2, was prepared.

For the third test a solution containing 13.8 mg/ml of lignin in a fosfate buffer, pH 10.7, was prepared.

15 For the fourth test a solution containing 12.9 mg/ml of lignin in a fosfate buffer, pH 9.6, was prepared.

To 1.8 ml of the above lignin solutions, 1.2 ml of enzyme solution diluted to 1000 PODU/ml with buffer were added. The test was incubated for 5, 10, and 15 minutes, respectively. After 5 and 10 minutes, respectively, 50 µl of 610 mM hydrogen 20 peroxide were added.

Average molecular weight determination was determined using HPLC, as a GPC-method.

The results of these tests are presented in Table 1, below.

Table 1Polymerization of Black Liquor™.

5	Blind	Average molecular weight			
		Incubation time			
		5 min.*	10 min.*	15 min	
	Test 1	13,500	15,700	18,800	18,400
	Test 2	13,200	63,400	238,000	269,000
10	Test 3	14,200	89,000	306,000	389,000
	Test 4	18,900	93,000	289,000	358,000

* Hydrogen peroxide added

CLAIMS

1. A method of enzymatic polymerization and/or modification of lignin or lignin containing material by treatment with a peroxidase, **characterized** in, that the enzymatic polymerization is performed at alkaline pH values, under a controlled addition of hydrogen peroxide, and that the polymerization is performed essentially in the absence of any organic solvents.
2. The method according to claim 1, in which the enzymatic polymerization is performed at a pH in the range of from 7-11, preferably in the range of 8-10.
3. The method of either of claims 1-2, in which the peroxidase is derived from Coprinus, e.g. C. cinerius or C. macrorhizus, or Bacillus, e.g. B. pumilus.
4. The method of any of claims 1-3, wherein the peroxidase dosage is in the range of 1-10,000 PODU per g of lignin, preferably 50-5,000 PODU per g of lignin.
5. A method of any of the preceding claims, in which the addition of hydrogen peroxide is administrated in a manner that keeps pace with consumption and/or degradation.
6. The method of claim 5, in which hydrogen peroxide is added discretely or continuously, either primary in the initial stage of the incubation period, or equally throughout the incubation period.
7. The method of either of claims 5-6, in which the addition of hydrogen peroxide is conducted manually or automatically, e.g. by use of a pump.
8. The method of claim 5, in which hydrogen peroxide is added enzymatically using a hydrogen peroxide generating

enzyme in the presence of its substrate, e.g. a glucose oxidase.

9. A method of any of the preceding claims, in which a hydrogen peroxide concentration in the range of 0.01-20 mM is maintained throughout the incubation period.

5 10. A method of any of the preceding claims, which method further comprises the addition of a chemical polymerizing agent, e.g. polydiphenylmethanediisocyanate (PMDI).

11. Use of the method according to any of claims 1-10 in the preparation of a glue or an active binder for use in the
10 manufacture of wood composites e.g. wood fibre materials such as chipboards, fibre boards, or particle boards, or in the manufacture of laminated wood products, such as laminated beams and plywood.

12. A glue or active binder obtained by subjecting
15 lignin sulphonate to the method of any of claims 1-10.

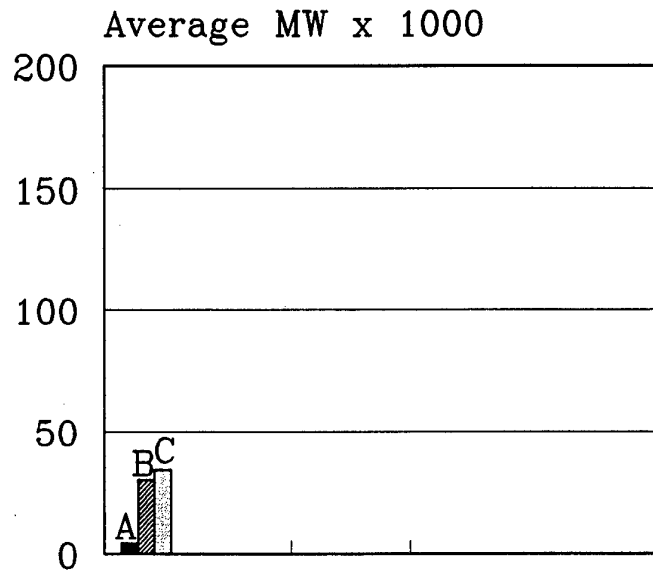


Fig. 1A

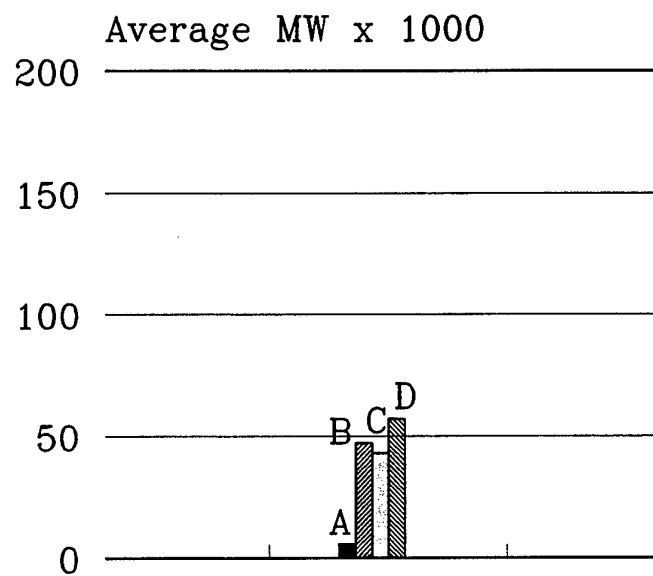


Fig. 1B

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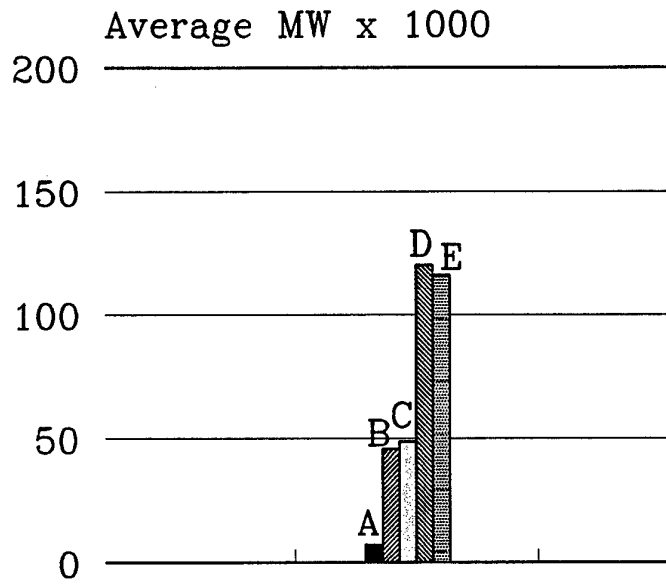


Fig. 1C

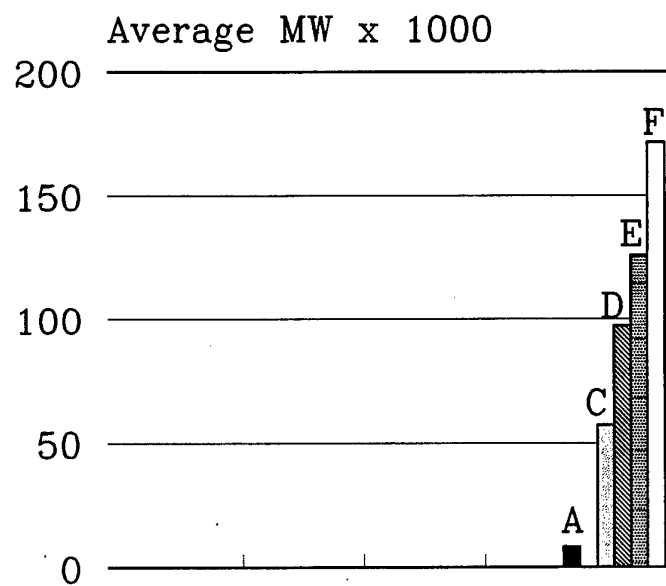


Fig. 1D

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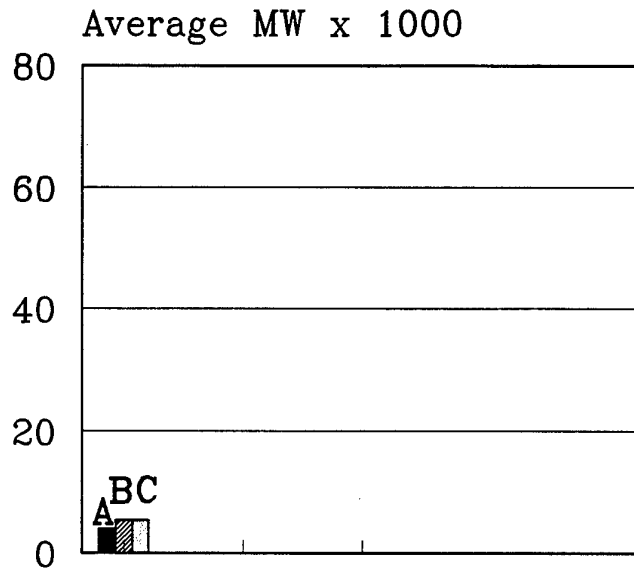


Fig. 2A

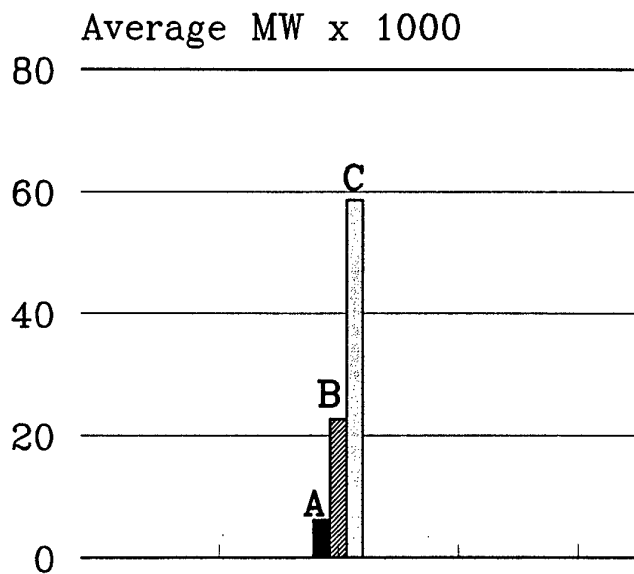


Fig. 2B

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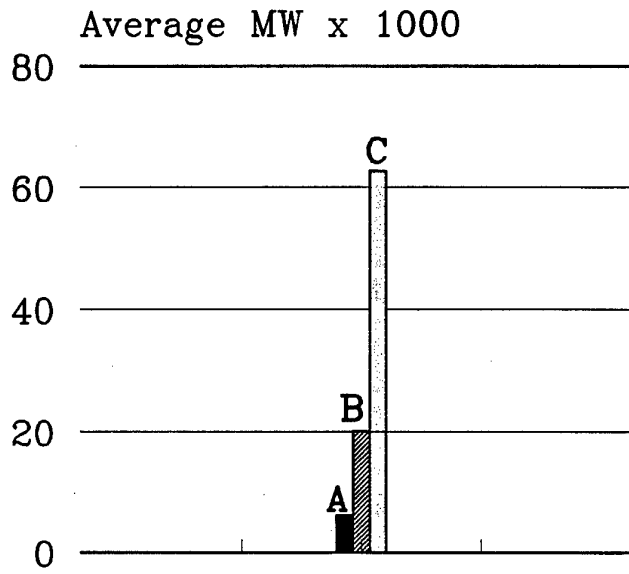


Fig. 2C

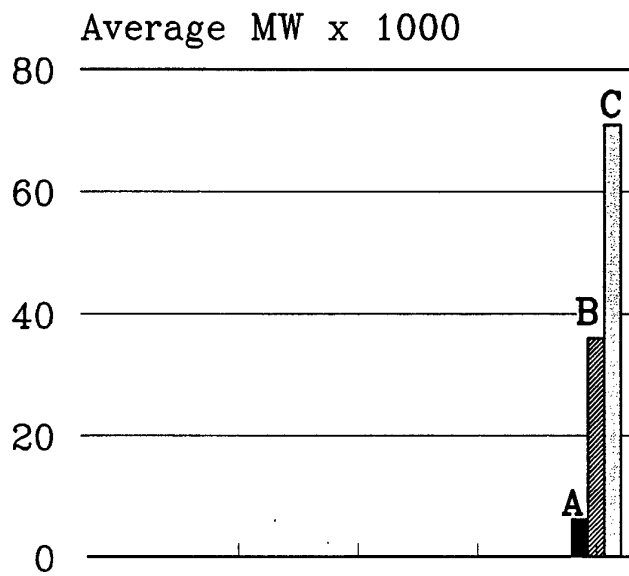


Fig. 2D

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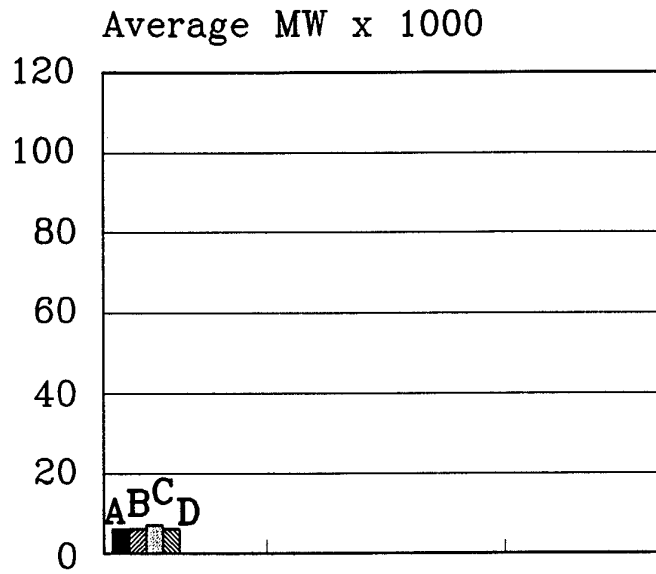


Fig. 3A

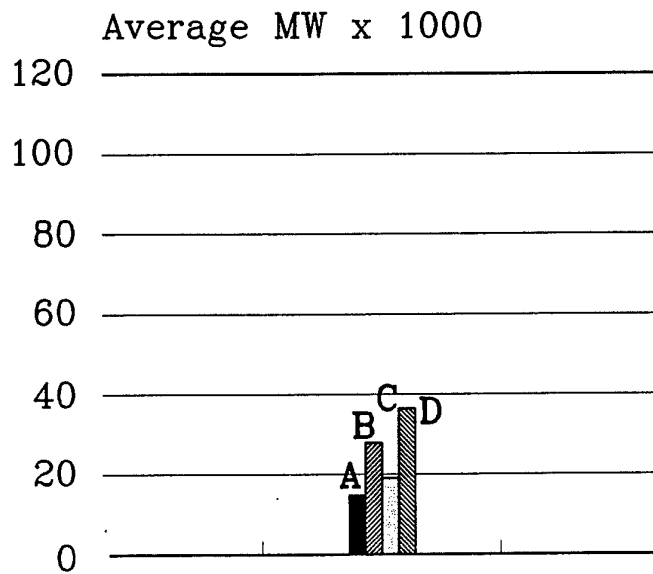


Fig. 3B

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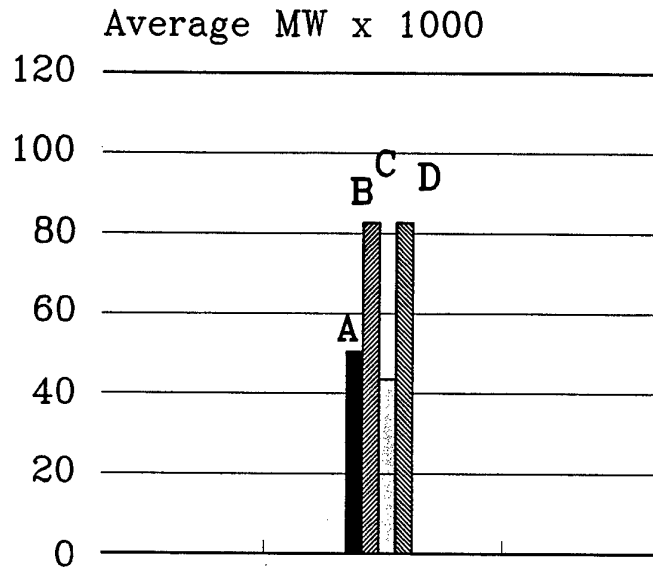


Fig. 3C

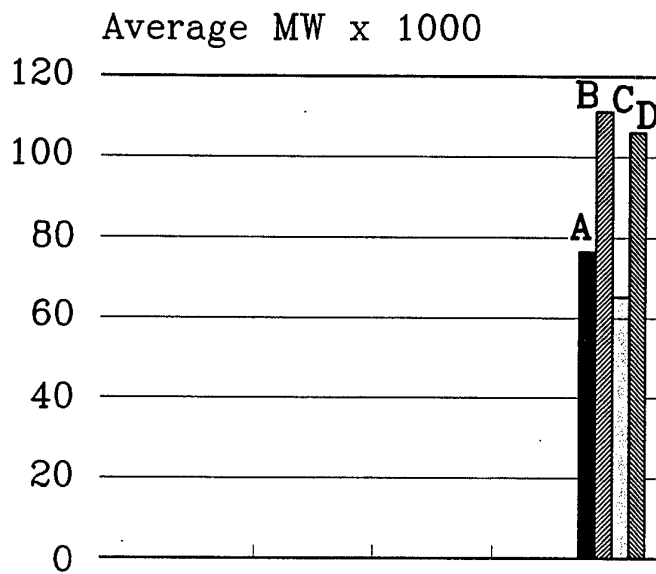


Fig. 3D

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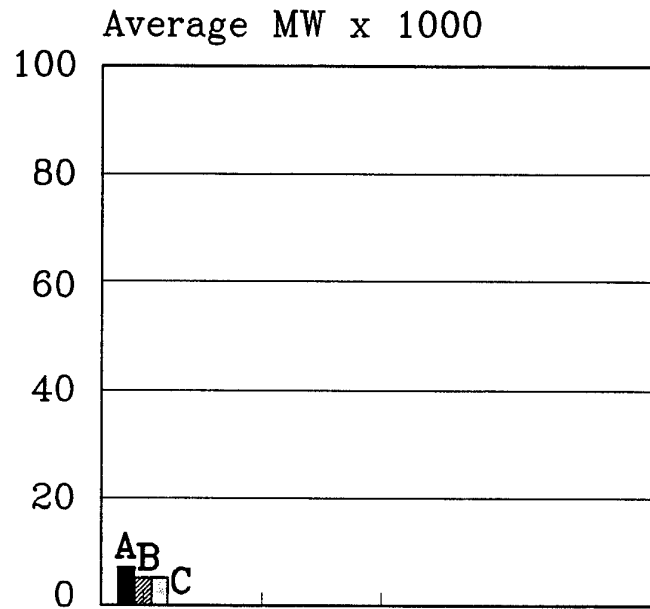


Fig. 4A

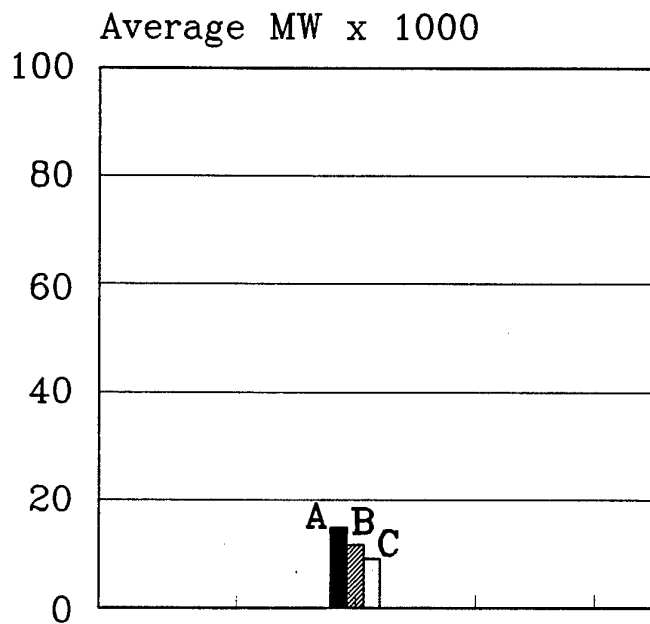


Fig. 4B

8/11

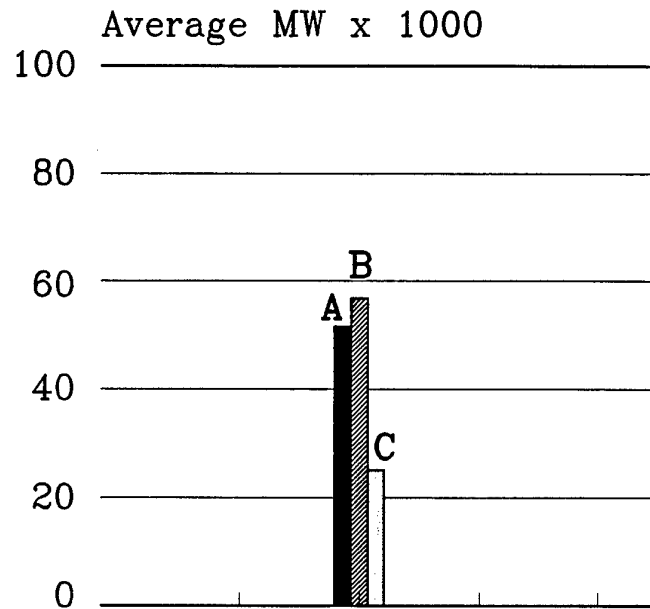


Fig. 4C

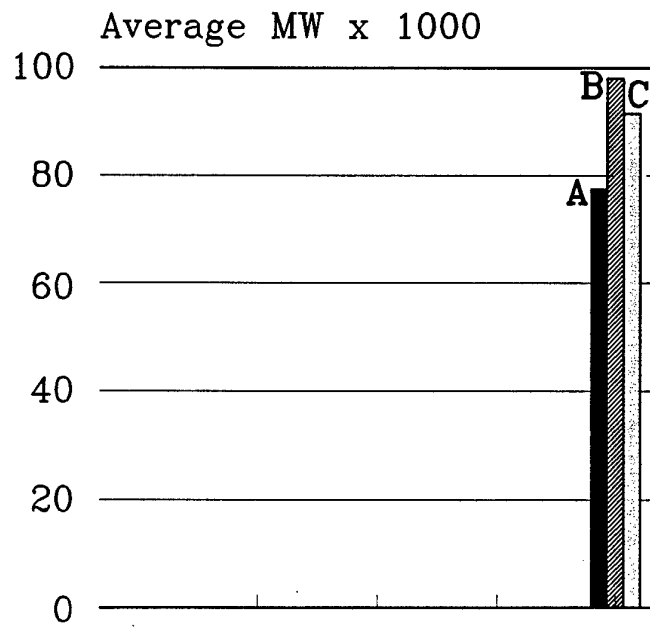


Fig. 4D

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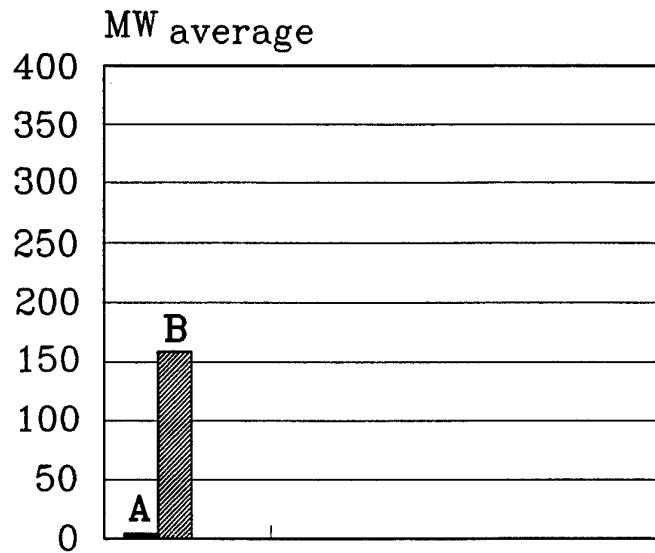


Fig. 5A

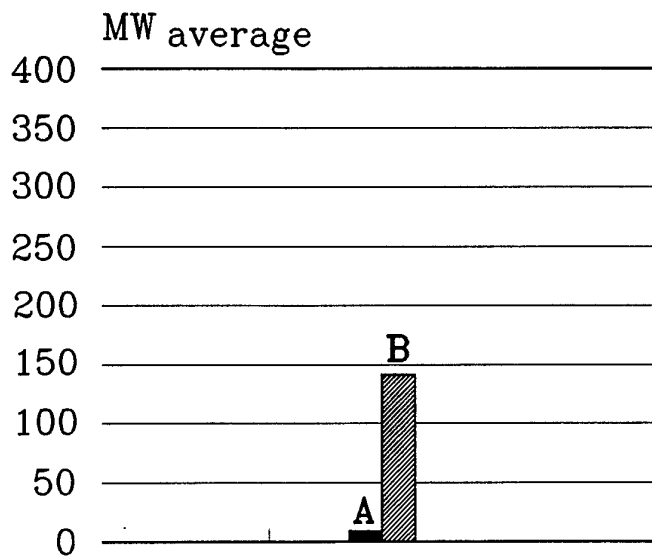


Fig. 5B

10/11

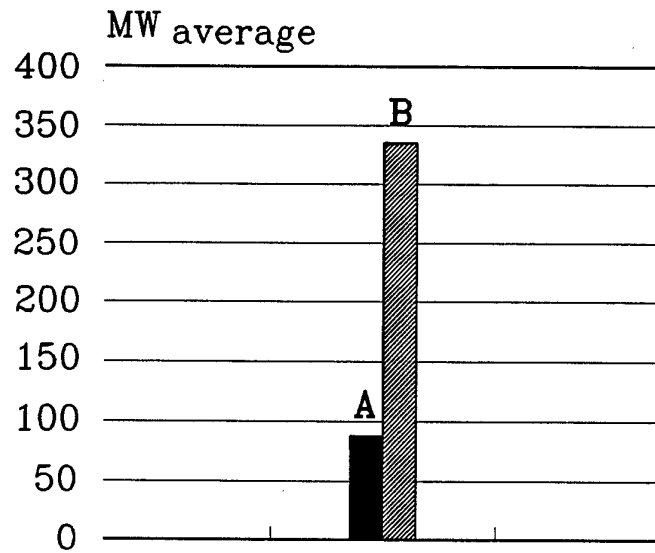


Fig. 5C

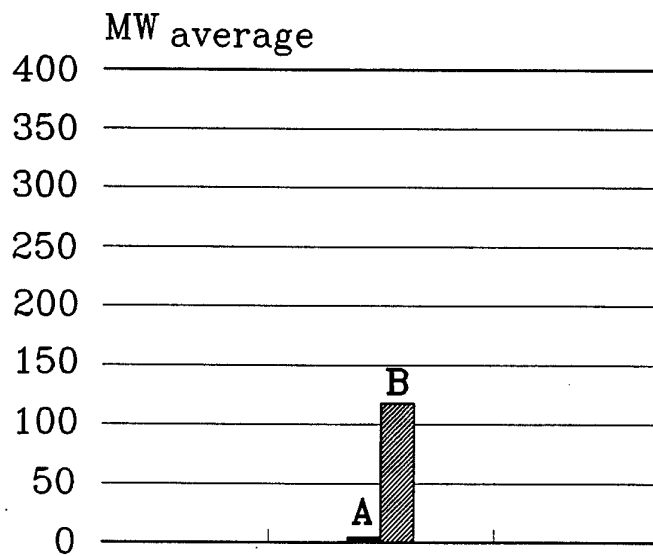


Fig. 5D

11/11

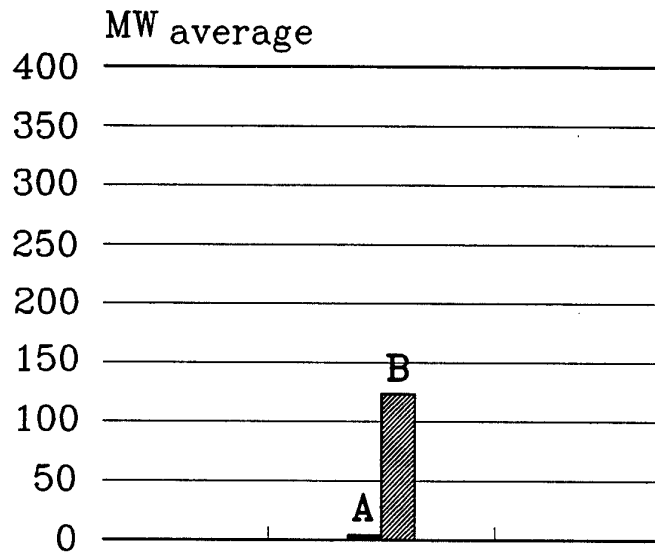


Fig. 5E

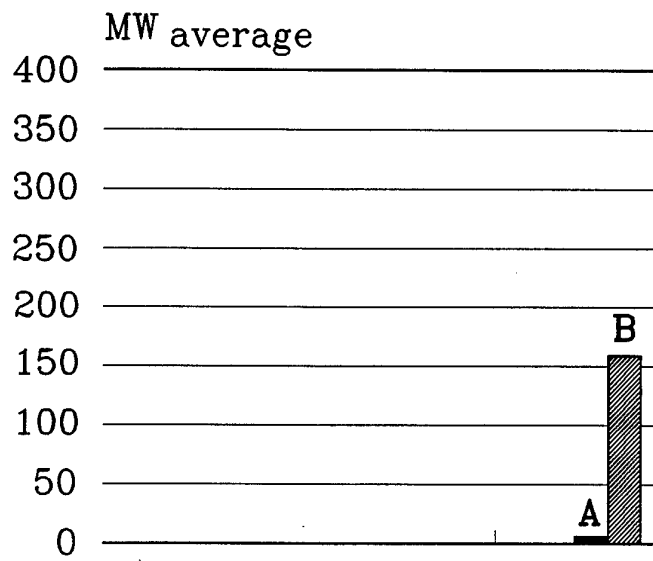


Fig. 5F

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 93/00217

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C08H 5/02, C08H 5/04, C07G 1/00, C08F 2/10
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C08H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, WPI, CLAIMS, US PATENT FULL TEXT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A2, 0354485 (HÜTTERMANN, ALOIS), 14 February 1990 (14.02.90)	1-12
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Further documents are listed in the continuation of Box C. See patent family annex.

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29 Sept 1993

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Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer
Jonny Brun
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

Information on patent family members

26/08/93

International application No.

PCT/DK 93/00217

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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