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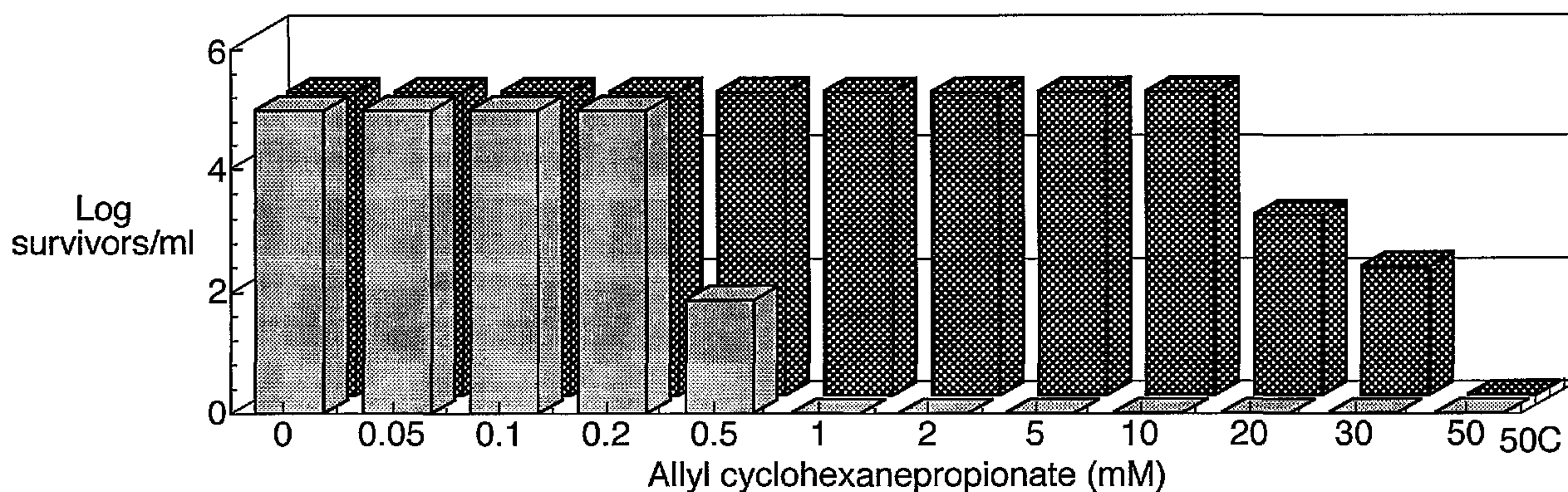
(72) Inventeurs/Inventors:
KIRBY, ROY MICHAEL, GB;
STEELS, HAZEL, GB;
STRATFORD, MALCOLM, GB

(73) Propriétaire/Owner:
UNILEVER PLC, GB

(74) Agent: RIDOUT & MAYBEE LLP

(54) Titre : BOISSON A STABILITE ELEVEE

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A method for preparing an ambient-stable beverage that is suitable for cold filling. The method involves adding to a beverage at least one pasteurisation adjunct that has no appreciable fungicidal activity at a temperature between 0 and 40 degrees C but exhibits fungicidal activity when heated to a temperature between 40 and 65 degrees C, and raising the temperature of the beverage to a temperature between 40 and 65 degrees C in order to activate the fungicidal activity of the pasteurisation adjunct.

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(71) Applicant (for AE, AG, AU, BB, BZ, CA, CY, GB, GD, GH, GM, IE, IL, KE, LC, LK, LS, MN, MW, NZ, SD, SG, SL, SZ, TT, TZ, UG, ZA, ZW only): **UNILEVER PLC** [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).

(71) Applicant (for AL, AM, AT, AZ, BA, BE, BF, BG, BJ, BR, BY, CF, CG, CH, CI, CM, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, FR, GA, GE, GN, GR, GW, HR, HU, ID, IS, IT, JP, KG, KP, KR, KZ, LR, LT, LU, LV, MA, MC, MD, MG, MK, ML, MR, MX, MZ, NE, NL, NO, PL, PT, RO, RU, SE, SI, SK, SN, TD, TG, TJ, TM, TR, UA, UZ, VN, YU only): **UNILEVER NV** [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).

(71) Applicant (for IN only): **HINDUSTAN LEVER LIMITED** [IN/IN]; Hindustan Lever House, 165/166 Backbay Reclamation, Maharashtra, 400 020 Mumbai (IN).

(72) Inventors: **KIRBY, Roy, Michael**; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). **STEELS, Hazel**; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB). **STRATFORD, Malcolm**; Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB).

(74) Agent: **EVANS, Jacqueline, Gail, Victoria**; Unilever PLC, Patent Department, Colworth House, Sharnbrook, Bedford, Bedfordshire MK44 1LQ (GB).

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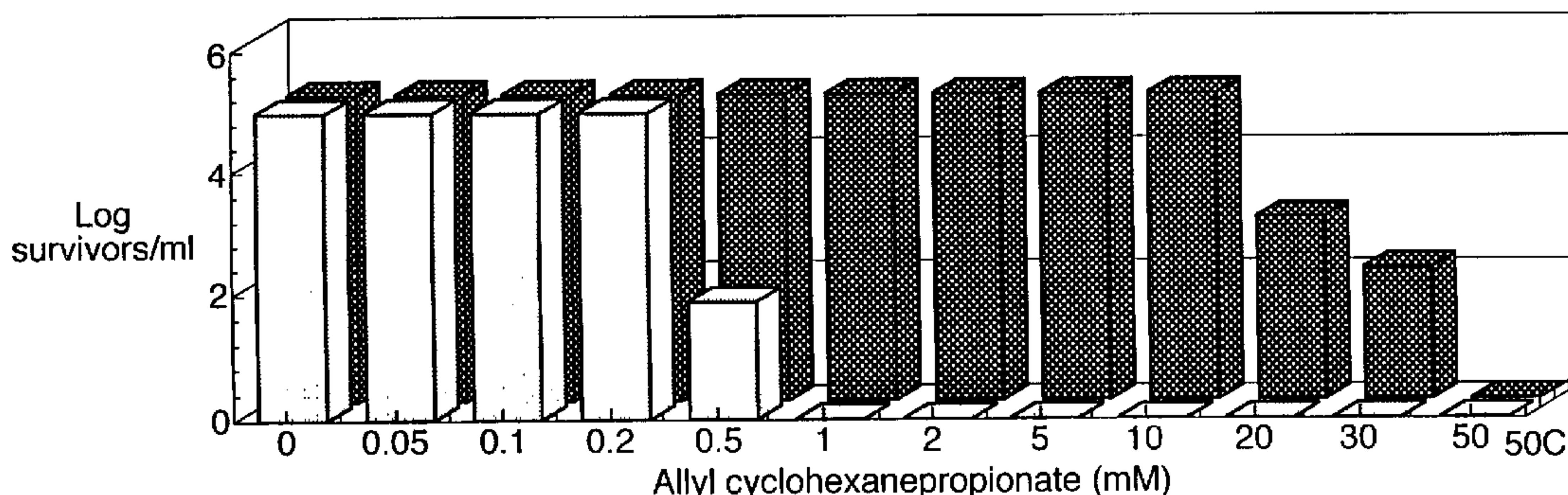
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(54) Title: AMBIENT STABLE BEVERAGE



(57) Abstract: A method for preparing an ambient-stable beverage that is suitable for cold filing. The method involves adding to a beverage at least one pasteurisation adjunct that has no appreciable fungicidal activity at a temperature between 0 and 40 degrees C but exhibits fungicidal activity when heated to a temperature between 40 and 65 degrees C, and raising the temperature of the beverage to a temperature between 40 and 65 degrees C in order to activate the fungicidal activity of the pasteurisation adjunct.



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AMBIENT STABLE BEVERAGE5 **Field of the invention**

The present invention relates to a method for preparing an ambient stable beverage, particularly a tea based beverage, that involves adding to the beverage one or more pasteurisation adjuncts that
10 become fungicidal when activated by heat.

Background and prior art

15 In recent years there has been an ever increasing choice for consumers who wish to quench their thirst with ready made beverages. Many of those are now turning from the well known soft drinks to tea based beverages, be those carbonated or still, and the "natural" refreshment they can provide.

20 Tea contains a complex combination of enzymes, biochemical intermediates and structural elements normally associated with plant growth and photosynthesis. There are also many natural substances that give tea its unique taste, astringency, aroma and
25 colour. Many of these are produced by the oxidation reactions that occur during the so-called fermentation stage of black tea manufacture. Tea production has long been driven by traditional processing methods with only a fundamental understanding of the chemistry that is involved. As a consequence manufacturers have
30 discovered making ambient stable tea based beverages at the volumes required to compete with more traditional soft drinks is not simply a matter of flavouring a soft drink with tea.

The flavour of a tea based beverage and its stability rely on the
35 stability of the beverage as a whole. The fungi including yeasts

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and moulds that can grow in tea based beverages and other soft drinks can be killed by heat treatment or at least controlled by use of preservatives. Some tea based beverages are therefore pasteurised and then bottled in glass or special heat stable PET containers. This is known as "hot filling". Unfortunately this can be an expensive operation that creates a great deal of environmentally unfriendly waste. It has therefore become more attractive for manufacturers to pack their tea based products in standard PET containers which can range from single serve units to multi-serve packs and maintain the stability of the product using tailor made flavour and preservative systems. This is known as "cold filling". It is also useful in that one can readily use a tea concentrate or powder.

Unfortunately the use of common preservatives can affect the flavour of a tea based beverage. This is particularly true for sulphite and sorbate. Adding a strong flavour such as lemon can offset the preservative taste. However consumers are keen to experience other flavours. Furthermore, some of those consumers that were drawn to tea based products as a more healthy and natural alternative to soft drinks sometimes view preservatives as the sort of synthetic additives they would rather avoid.

Many countries have regulations that prohibit the use of certain food additives, including some fungicides and preservatives, in foods and beverages. Regulations can vary widely but there is a clear trend for foods to contain fewer and lower levels of chemical fungicides and preservatives, particularly synthetic ones.

Accordingly there is a need for a method for preparing pleasantly flavoured ambient-stable beverages that have low levels of synthetic preservatives.

In response to that need the present inventors have now developed a fungicidal system for tea based beverages that does not contain

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any synthetic fungicides or preservatives. The fungicidal system can also be used to stabilise non-tea based beverages including fruit and soft drinks.

5

Statement of the Invention

The invention can in broad terms be said to relate to a method for preparing an ambient-stable beverage suitable
10 for cold filling comprising the steps of: adding to a beverage at least one pasteurisation adjunct that has no appreciable fungicidal activity at a temperature between 0 and 40 degrees C but exhibits fungicidal activity when heated to a temperature between 40 and 65 degrees C, and
15 raising the temperature of the beverage to a temperature between 40 and 65 degrees C in order to activate the fungicidal activity of the pasteurisation adjunct. When the beverage is tea based it preferably contains 0.01 to 3% tea solids, especially about 0.14% tea solids.

20

The pasteurisation adjunct is preferably a substance that has no appreciable fungicidal activity at a temperature between 0 and 40 °C, especially between 20 and 35 °C, but exhibits fungicidal activity when heated to a temperature between 40 and 65 °C,
25 especially between 45 and 55 °C.

Particularly preferred pasteurisation adjuncts include decyl acetate, lauric acid, lauric aldehyde, lauric alcohol, 2-dodecenal, ethyl 2-decenoate, geranyl acetone, geranyl acetate
30 which are preferably present in a concentration no greater than 1 mM, preferably no greater than 0.1 mM.

Unlike pasteurisation-based methods for stabilising beverages, the present method is not time or temperature dependent.

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"Beverage" for the purposes of the present invention means any drink, other than water, and includes soft drinks, fruit drinks, coffee based drinks and tea based drinks.

5 "Tea" for the purposes of the present invention means leaf material from *Camellia sinensis* var. *sinensis* or *Camellia sinensis* var. *assamica*. "Tea" is also intended to include the product of blending two or more of any of these teas.

10 For the avoidance of doubt the word "comprising" is intended to mean including but not necessarily "consisting of" or "composed of". In other words the listed steps or options need not be exhaustive.

15 Except in the operating and comparative examples, or where otherwise explicitly indicated, all numbers in this description indicating amounts or concentrations of material ought to be understood as modified by the word "about".

20

Brief description of the drawings

Figure 1 shows pasteurisation adjunct, decyl acetate, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea.

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Figure 2 shows pasteurisation adjunct, lauric acid, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea.

30 Figure 3 shows pasteurisation adjunct, lauric alcohol, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea.

Figure 4 shows pasteurisation adjunct, lauric aldehyde, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea.

35 Figure 5 shows pasteurisation adjunct, geranyl acetate, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea.

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Figure 6 shows pasteurisation adjunct, geranyl acetone, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea.

Figure 7 shows pasteurisation adjunct, ethyl-2-decenoate, applied
5 at 0-200 ppm in Ready to Drink tea, 0.14% tea.

Figure 8 shows a 9:1 mixture of pasteurisation adjuncts, decyl acetate:lauric acid, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea.

10

Figure 9 shows the effect of pasteurisation adjuncts applied at 0-100 ppm in synthetic soft drink, zero tea content.

Figure 10 shows the effect on bacteria of heat + pasteurisation
15 adjuncts, decyl acetate or lauric acid, applied at 0-100 ppm in Ready to Drink tea, 0.14% tea or synthetic soft drink.

Figure 11 is a Scatter plot of molecular weight/partition coefficient of compounds able to function as pasteurisation
20 adjuncts.

Figures 12 to 47 show the high effect pasteurisation adjuncts listed in Table 1 tested in YEPD broth pH 3.4.

25 Figures 48 to 63 show the moderate effect pasteurisation adjuncts listed in Table 1 tested in YEPD broth pH 3.4.

Figures 64 to 77 show the low effect pasteurisation adjuncts listed in Table 1 tested in YEPD broth pH 3.4.

30

Figures 78 to 82 show the control compounds showing little effect as pasteurisation adjuncts tested in YEPD broth pH 3.4.

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Detailed description of the invention

The present invention relates to a method for preparing an ambient stable beverage. The method involves adding to a beverage at
5 least one pasteurisation adjunct that has no appreciable fungicidal activity at a temperature between 0 and 40 degrees C but exhibits fungicidal activity when heated to a temperature between 40 and 65 degrees C, and raising the temperature of the beverage to a temperature between 40 and 65 degrees C in order to
10 activate the fungicidal activity of the pasteurisation adjunct.

Pasteurisation describes the process of inactivating enzymes and reducing the population of micro-organisms that occurs when a beverage is heated to a minimum temperature between 62.5 and 100
15 °C for a given period. One obtains a better pasteurisation by using higher temperatures and longer treatment times. In contrast to this the present invention arises from the finding that certain chemical substances that are not generally regarded as having any appreciable fungicidal activity at or near room temperature do in
20 fact exhibit fungicidal activity when they are heated to about 50 °C. This means that beverages containing such compounds can be heated to a temperature somewhat below 65 °C and yet the micro-organism population can be reduced to levels that one would expect to achieve by pasteurisation. Hence the chemical compounds can be
25 described as pasteurisation adjuncts. But unlike pasteurisation-based methods for stabilising beverages, the performance of the present method is not time or temperature dependent.

The present inventors initially found that the compounds such as
30 decyl acetate, lauric acid, and methyl decenoate have no fungicidal activity at 30 °C and yet they exhibit significant fungicidal activity when heated to 50 °C.

This led them to test other compounds that have no fungicidal
35 activity at 30 °C. They found that the compounds listed in Table

1 below do indeed exhibit significant fungicidal activity when heated
 to 50 °C. In the table the minimum inhibitory concentration (MIC) is
 given for each compound as well as the molecular weight (M.W.), the
 logP_{oct} and a rating of its importance. A compound is regarded as being
 5 of high importance if a low concentration is required to exhibit a
 substantial fungicidal activity. The logP_{oct}, that is the logarithm of
 the partition coefficient of the relevant compound in octanol and thus
 a measure of its fungicidal activity, was determined using CHEMDRAW™
 software from the CHEMOFFICE ULTRA ENHANCED 2000™ software package
 10 (version 5.5) that is commercially available from CambridgeSoft.

TABLE 1

15 Compounds that exhibit fungicidal
 activity at 50 °C but not at 30 °C

	<u>COMPOUND</u>	<u>MIC50 (mM)</u>	<u>M.W.</u>	<u>logP_{oct}</u>	<u>Importance</u>
	Allyl cyclohexanepropionate	1	196	3.49	High
20	Amyl hexanoate	2	186	3.88	High
	Amyl octanoate	60	214	4.79	Mod
	Benzoin	10	212	2.53	10
	Benzyl benzoate	20	212	3.00	Mod
	Benzyl salicylate	50	228	2.61	Low
25	Bornyl acetate	2	196	2.66	Low
	Butyl heptanoate	5	186	3.88	High
	Butyl laurate	100	256	6.16	Low
	Butyl 10-undecenoate	40	240	5.46	Mod
	Carvyl propionate	5	208.3	2.81	Mod
30	β-Caryophyllene	70	204	-	Low
	Decyl acetate	1	200	4.34	High
	Decyl butyrate	70	228	5.25	Low
	Decyl propionate	50	214.35	4.79	High
	2-Dodecenal	<0.05 (9 ppm)	182	4.44	High
35	Ethyl decanoate	40	200	4.34	High
	Ethyl 2-decenoate	1	198	4.16	High
	Ethyl laurate	80	228	5.25	Mod
	Ethyl nonanoate	10	186	3.88	High
	Ethyl tridecanoate	100	242	5.71	Low

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	Ethyl undecanoate	50	214	4.79	Mod
	Ethyl 10-undecenoate	50	212	4.55	High
	Geranyl acetate	1	196	-	High
	Geranylacetone	0.5	194	-	High
5	Geranyl butyrate	40	224	-	High
	Geranyl propionate	12	210	-	High
	Heptyl butyrate	2	186	3.88	High
	w-6-Hexadecalactone	50	252	-	Low
	Hexadecanol	100	242	6.48	Low
10	Hexyl hexanoate	30	201	4.34	High
	Hexyl octanoate	10	228	5.25	Mod
	Isoamyl hexanoate	5	186	3.63	High
	Isoamyl laurate	120	270	6.37	Low
	Isoamyl salicylate	15	208	2.71	Mod
15	Lauric acid	0.1 (20 ppm)	200	4.49	High
	Lauric alcohol	0.1 (18.6ppm)	186	4.66	High
	Lauric aldehyde	0.2 (36 ppm)	184	4.61	High
	Lauryl acetate	40	228	5.25	Mod
	Linalyl acetate	10	196	2.78	Mod
20	Linalyl propionate	20	210	3.43	Mod
	Methyl decanoate	2	186	4.05	High
	Methyl laurate	40	214	4.96	High
	Methyl myristate	60	242	5.88	Low
	Methyl nonanoate	2	172	3.6	High
25	Methyl undecanoate	50	200	4.51	High
	Methyl-9-undecenoate	40	198	3.79	High
	Myristaldehyde	40	212	5.53	Mod
	Myristic acid	50	228	5.41	High
	Nerolidol	1.5	222	4.08	High
30	Neryl butyrate	50	224	2.88	Mod
	Neryl isobutyrate	30	224	3.94	High
	Nonyl acetate	5	186	3.88	High
	Octyl butyrate	40	200	4.34	High
	w-Pentadecalactone	60	240	-	Low
35	Pentadecanoic acid	80	242	5.86	Low
	Pentadecanol	75	228	6.03	Low
	Phenethyl hexanoate	10	220	3.99	High
	Phenethyl octanoate	40	248	4.90	Mod
	2-Phenoxyethyl isobutyrate	0.1	226	5.04	High

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	Tetradecanol	50	214	5.57	Mod
	Tridecanal	5	200	5.07	High
	Tridecanoic acid	0.2	214	4.95	High
5	Tridecanol	0.05	198	5.11	High
	2-Tridecenal	<0.05 (9.8ppm)	196	4.90	High
	2-Undecanone	1	170	3.68	High

10 Many so-called pasteurisation adjuncts will provide effective fungicidal activity when heated to 50 °C or some other temperature below 65 °C. However certain compounds may prove to be more

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appropriate than others in terms of their effect on the taste of the tea based beverage. Accordingly the present inventors have identified the following compounds as being preferred pasteurisation adjuncts for use in the method of the invention:

5 allyl cyclohexanepropionate, amyl hexanoate, butyl heptanoate, decyl acetate, decyl propionate, 2-dodecenal, ethyl decanoate ethyl 2-decenoate, ethyl nonanoate, ethyl 10-undecenoate, geranyl acetate, geranylacetone, geranyl butyrate, geranyl propionate, heptyl butyrate, hexyl hexanoate, isoamyl hexanoate, lauric acid,
10 lauric alcohol, lauric aldehyde, methyl decanoate, methyl laurate, methyl nonanoate, methyl undecanoate, methyl-9-undecenoate, myristic acid, nerolidol, neryl isobutyrate, nonyl acetate, octyl butyrate, phenethyl hexanoate, 2-phenoxyethyl isobutyrate, tridecanal, tridecanoic acid, tridecanol, 2-tridecenal and
15 2-undecanone. The pasteurisation adjuncts are preferably present in a concentration no greater than 1 mM, and especially no greater than 0.1 mM.

From that list decyl acetate, lauric acid, lauric aldehyde, lauric
20 alcohol, 2-dodecenal, ethyl 2-decenoate, geranyl acetone, geranyl acetate are particularly preferred.

While not wanting to be bound by theory, the mode of fungicidal action of these pasteurisation adjuncts in at least tea based
25 beverages is believed to involve disruption of microbial membranes. It is thought that at high temperatures these compounds are able to enter into membranes and cause microbial death by cell lysis.

30 The inventors postulated that the aforementioned compounds might not be the only compounds that might function as pasteurisation adjuncts in this way. One might define effective pasteurisation adjuncts on the basis of their Quantitative Structure Activity Relationship (QSAR) parameters. This definition would include
35 known and hitherto unknown chemical compounds. The full list of the pasteurisation adjuncts tested above were plotted in respect

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of their molecular weight and logP_{oct} values in Figure 11. From that Figure the inventors forecast that preferred pasteurisation adjuncts can be defined as those that have a molecular weight between 170 and 230 daltons and a logP_{oct} value between 3.5 and 5.5.

Water quality can seriously undermine the stability of a beverage. This is an important factor when making a beverage, particularly a tea based beverage, for cold filling. For that purpose it will often be important to minimise the yeast content of water used at all stages of production. Art known methods include chlorination/dechlorination and UV irradiation.

Ambient-stable beverages made by the method of the invention may be still or carbonated. Carbonation appears to provide a preservative effect in itself and therefore the formulation of a carbonated product need not be the same as a still one.

Tea is known to have certain antibacterial and antiviral properties in itself. One must exceed a concentration of about 3% to evidence tea beginning to suppress the growth of yeasts and moulds. At concentrations lower than this, which is typical for tea based beverages, tea acts as a nutrient that enhances the potential for microbial spoilage.

Most microbes that can typically grow in tea based beverages thrive on sugar, a source of nitrogen, oxygen, zinc, magnesium, potassium, phosphate and vitamins. It is therefore advantageous to limit the sugar content to 8 to 10 degrees brix, however one could use up to 60 degrees brix when the product is a tea mix. Oxygen content can be minimised by pre-pasteurisation or some heat treatment or nitrogen sparging. The mineral content of a tea based beverage can be minimised using EDTA, citrate, or a water softener. For example microbes can grow in tea if the concentration of magnesium ions exceeds 0.2 ppm, and they only

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need trace levels of zinc. One must be careful using citrate for this purpose as it can affect taste.

The invention will now be described in the following examples with
5 reference to the accompanying drawings.

EXAMPLE 1

Ready to Drink tea experiments

10

Figure 1 shows pasteurisation adjunct, decyl acetate, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before
15 inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at
20 600 nm in x11 diluted samples. The pasteurisation adjunct had little effect at low temperature on yeast growth as did heat up to 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

25

Figure 2 shows pasteurisation adjunct, lauric acid, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before
inoculation with 10^4 cells/ml of the yeast *Saccharomyces*
30 *cerevisiae* X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had
35 little effect at low temperature on yeast growth as did heat up to

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60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

Figure 3 shows pasteurisation adjunct, lauric alcohol, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had little effect at low temperature on yeast growth as did heat up to 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

Figure 4 shows pasteurisation adjunct, lauric aldehyde, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had little effect at low temperature on yeast growth as did heat up to 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

Figure 5 shows pasteurisation adjunct, geranyl acetate, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces*

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cerevisiae X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had little effect at low temperature on yeast growth as did heat up to 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

10 Figure 6 shows pasteurisation adjunct, geranyl acetone, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces*
15 *cerevisiae* X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had
20 little effect at low temperature on yeast growth as did heat up to 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

Figure 7 shows pasteurisation adjunct, ethyl-2-decenoate, applied
25 at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces*
cerevisiae X2180-1B. Tubes were held at temperature for 2 minutes
30 before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had little effect at low temperature on yeast growth as did heat up to
35 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

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Figure 8 shows a 9:1 mixture of pasteurisation adjuncts, decyl acetate:lauric acid, applied at 0-200 ppm in Ready to Drink tea, 0.14% tea. Rows of 30 ml tubes containing 10 ml RTD tea, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had little effect at low temperature on yeast growth as did heat up to 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

EXAMPLE 2

Synthetic soft drink experiments

Figure 9 shows the effect of pasteurisation adjuncts applied at 0-100 ppm in synthetic soft drink, zero tea content. Synthetic soft drink contained glucose, 8%w/v, citric acid 3 g/l, potassium orthophosphate 1 g/l, magnesium chloride 0.1 g/l and yeast extract 0.1 g/l. Rows of 30 ml tubes containing 10 ml synthetic soft drink, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving yeasts to grow out. At 14 days growth was measured by optical density at 600 nm in x11 diluted samples. The pasteurisation adjunct had little effect at low temperature on yeast growth. 50°C temperature + pasteurisation adjuncts showed a remarkable combined synergistic effect.

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EXAMPLE 3**Bacteria experiments**

Figure 10 shows the effect on bacteria of heat + pasteurisation adjuncts, decyl acetate or lauric acid, applied at 0-100 ppm in Ready to Drink tea, 0.14% tea or synthetic soft drink. Rows of 30 ml tubes containing 10 ml soft drink, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the bacteria, *Gluconobacter sp.* 222. Tubes were held at temperature for 2 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 14 days at 25°C to allow surviving bacteria to grow out. At 14 days growth was measured visually. The pasteurisation adjunct had little effect at low temperature on yeast growth as did heat up to 60°C, without pasteurisation adjunct. Heat + pasteurisation adjunct showed a remarkable combined synergistic effect.

EXAMPLE 4**Identification of preferred pasteurisation adjuncts**

Figure 11 is a Scatter plot of molecular weight/partition coefficient of compounds able to function as pasteurisation adjuncts. The compounds are listed on Table 1, together with logPoct (partition coefficient) and molecular weight (M.W.) Compounds were assessed as High, Moderate or Low as pasteurisation adjuncts. High effects compounds, most preferred, generally showed at least a 5 fold decrease in MIC, minimum inhibitory concentration at 50°C compared with 30°C.

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EXAMPLE 5**Experiments with high effect pasteurisation adjuncts**

Figures 12 to 47 show the high effect pasteurisation adjuncts listed in Table 1 tested in YEPD broth pH 3.4. YEPD contained glucose, 20 g/l, peptone 20 g/l and yeast extract 10 g/l. Rows of 30 ml tubes containing 10 ml YEPD, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 10 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 48 hours at 25°C to allow surviving yeasts to grow out, and form discrete colonies. The pasteurisation adjunct had little effect at low temperature on yeast survival. 50°C temperature + pasteurisation adjuncts showed a remarkable combined synergistic effect, acting to rapidly kill the yeast inoculum.

EXAMPLE 6**Experiments with moderate effect pasteurisation adjuncts**

Figures 48 to 63 show the moderate effect pasteurisation adjuncts listed in Table 1 tested in YEPD broth pH 3.4. YEPD contained glucose, 20 g/l, peptone 20 g/l and yeast extract 10 g/l. Rows of 30 ml tubes containing 10 ml YEPD, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 10 minutes before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 48 hours at 25°C to allow surviving yeasts to grow out, and form discrete colonies. The pasteurisation adjunct had little effect at low temperature on yeast survival. 50°C temperature + pasteurisation adjuncts showed a remarkable

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combined synergistic effect, acting to rapidly kill the yeast inoculum.

5 **EXAMPLE 7**

Experiments with low effect pasteurisation adjuncts

Figures 64 to 77 show the low effect pasteurisation adjuncts listed in Table 1 tested in YEPD broth pH 3.4. YEPD contained
10 glucose, 20 g/l, peptone 20 g/l and yeast extract 10 g/l. Rows of 30 ml tubes containing 10 ml YEPD, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 10 minutes
15 before being cooled rapidly in cold water for 5 minutes. Tubes were then incubated for 48 hours at 25°C to allow surviving yeasts to grow out, and form discrete colonies. The pasteurisation adjunct had little effect at low temperature on yeast survival. 50°C temperature + pasteurisation adjuncts showed a remarkable
20 combined synergistic effect, acting to rapidly kill the yeast inoculum.

EXAMPLE 8

25 **Control experiments**

Figures 78 to 82 show the control compounds showing little effect as pasteurisation adjuncts tested in YEPD broth pH 3.4. YEPD contained glucose, 20 g/l, peptone 20 g/l and yeast extract 10
30 g/l. Rows of 30 ml tubes containing 10 ml YEPD, pH 3.4, were equilibrated at the required temperature for 7 minutes in a water bath, before inoculation with 10^4 cells/ml of the yeast *Saccharomyces cerevisiae* X2180-1B. Tubes were held at temperature for 10 minutes before being cooled rapidly in cold water for 5
35 minutes. Tubes were then incubated for 48 hours at 25°C to allow

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surviving yeasts to grow out, and form discrete colonies. Control compounds had a normal effect at low temperature on yeast survival and at 50°C temperature showed only very slight improvements in activity.

CLAIMS:

1. A method for preparing an ambient-stable beverage
suitable for cold filing comprising the steps of:
5 adding to a beverage at least one pasteurisation adjunct
that has no appreciable fungicidal activity at a temperature
between 0 and 40 degrees C but exhibits fungicidal activity
when heated to a temperature between 40 and 65 degrees C,
10 and raising the temperature of the beverage to a temperature
between 40 and 65 degrees C in order to activate the
fungicidal activity of the pasteurisation adjunct.
- 15 2. A method according to claim 1, wherein the
pasteurisation adjunct has no appreciable fungicidal
activity at a temperature between 20 and 35 °C.
- 20 3. A method according to claim 2, wherein the
pasteurisation adjunct exhibits fungicidal activity when
heated to a temperature between 45 and 55 °C.
- 25 4. A method according to any one of claims 1 to 3, wherein
the pasteurisation adjunct is selected from the group
consisting of allyl cyclohexanepropionate, amyl hexanoate,
amyl octanoate, benzoin, benzyl benzoate, benzyl salicylate,
bornyl acetate, butyl heptanoate, butyl laurate, butyl 10-
undecenoate, carvyl propionate, β -caryophyllene, decyl
acetate, decyl butyrate, decyl propionate, 2-dodecenal,
30 ethyl decanoate, ethyl 2-decenoate, ethyl laurate, ethyl
nonanoate, ethyl tridecanoate, ethyl undecanoate, ethyl 10-
undecenoate, geranyl acetate, geranylacetone, geranyl
butyrate, geranyl propionate, heptyl butyrate, w-6-
hexadecalactone, hexadecanol, hexyl hexanoate, hexyl
35 octanoate, isoamyl hexanoate, isoamyl laurate, isoamyl

salicylate, lauric acid, lauric alcohol, lauric aldehyde,
lauryl acetate, linalyl acetate, linalyl propionate, methyl
decanoate, methyl laurate, methyl myristate, methyl
nonanoate, methyl undecanoate, methyl-9-undecenoate,
5 myristaldehyde, myristic acid, nerolidol, neryl butyrate,
neryl isobutyrate, nonyl acetate, octyl butyrate, w-
pentadecalactone, pentadecanoic acid, pentadecanol,
phenethyl hexanoate, phenethyl octanoate, 2-phenoxyethyl
isobutyrate, tetradecanol, tridecanal, tridecanoic acid,
10 tridecanol, 2-tridecenal and 2-undecanone.

5. A method according to claim 4, wherein the
pasteurisation adjunct is selected from the group consisting
of allyl cyclohexanepropionate, amyl hexanoate, butyl
15 heptanoate, decyl acetate, decyl propionate, 2-dodecenal,
ethyl decanoate ethyl 2-decenoate, ethyl nonanoate, ethyl
10-undecenoate, geranyl acetate, geranylacetone, geranyl
butyrate, geranyl propionate, heptyl butyrate, hexyl
hexanoate, isoamyl hexanoate, lauric acid, lauric alcohol,
20 lauric aldehyde, methyl decanoate, methyl laurate, methyl
nonanoate, methyl undecanoate, methyl-9-undecenoate,
myristic acid, nerolidol, neryl isobutyrate, nonyl acetate,
octyl butyrate, phenethyl hexanoate, 2-phenoxyethyl
isobutyrate, tridecanal, tridecanoic acid, tridecanol, 2-
25 tridecenal and 2-undecanone.

6. A method according to claim 5, wherein the
pasteurisation adjunct is selected from the group consisting
of decyl acetate, lauric acid, lauric aldehyde, lauric
30 alcohol, 2-dodecenal, ethyl 2-decenoate, geranyl acetone and
geranyl acetate.

7. A method according to any one of claims 1 to 6, wherein
the pasteurisation adjunct is a compound that has a

molecular weight between 170 and 230 daltons and a logP_{oct} value between 3.5 and 5.5.

8. A method according to any one of claims 1 to 7, wherein
5 the concentration of the pasteurisation adjunct is no greater than 1 mM.

9. A method according to claim 8, wherein the
concentration of the pasteurisation adjunct is no greater
10 than 0.1 mM.

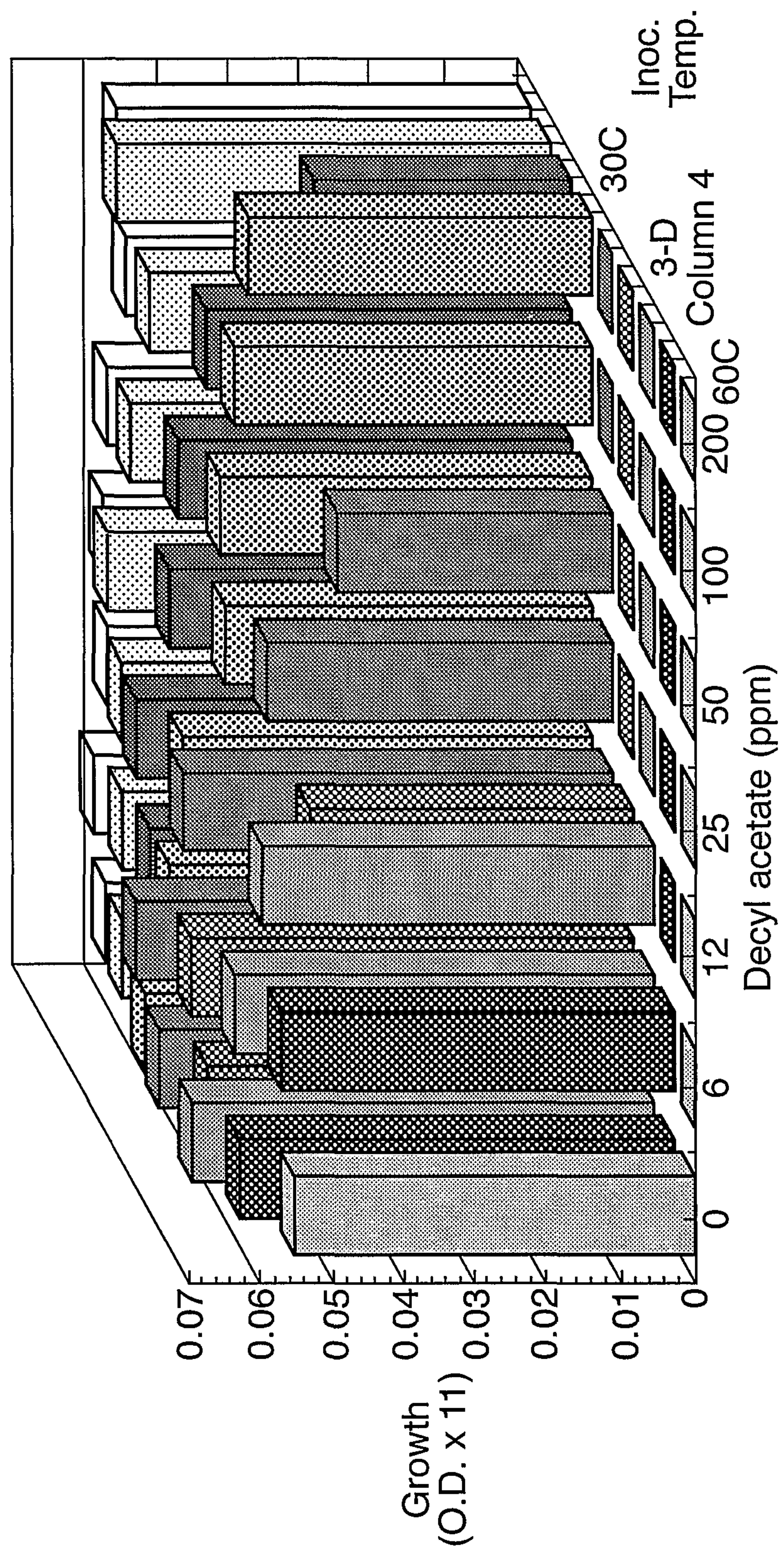
10. A method according to any one of claims 1 to 9, wherein the beverage is tea based.

15 11. A method according to claim 10, wherein the beverage contains 0.01 to 3% tea solids.

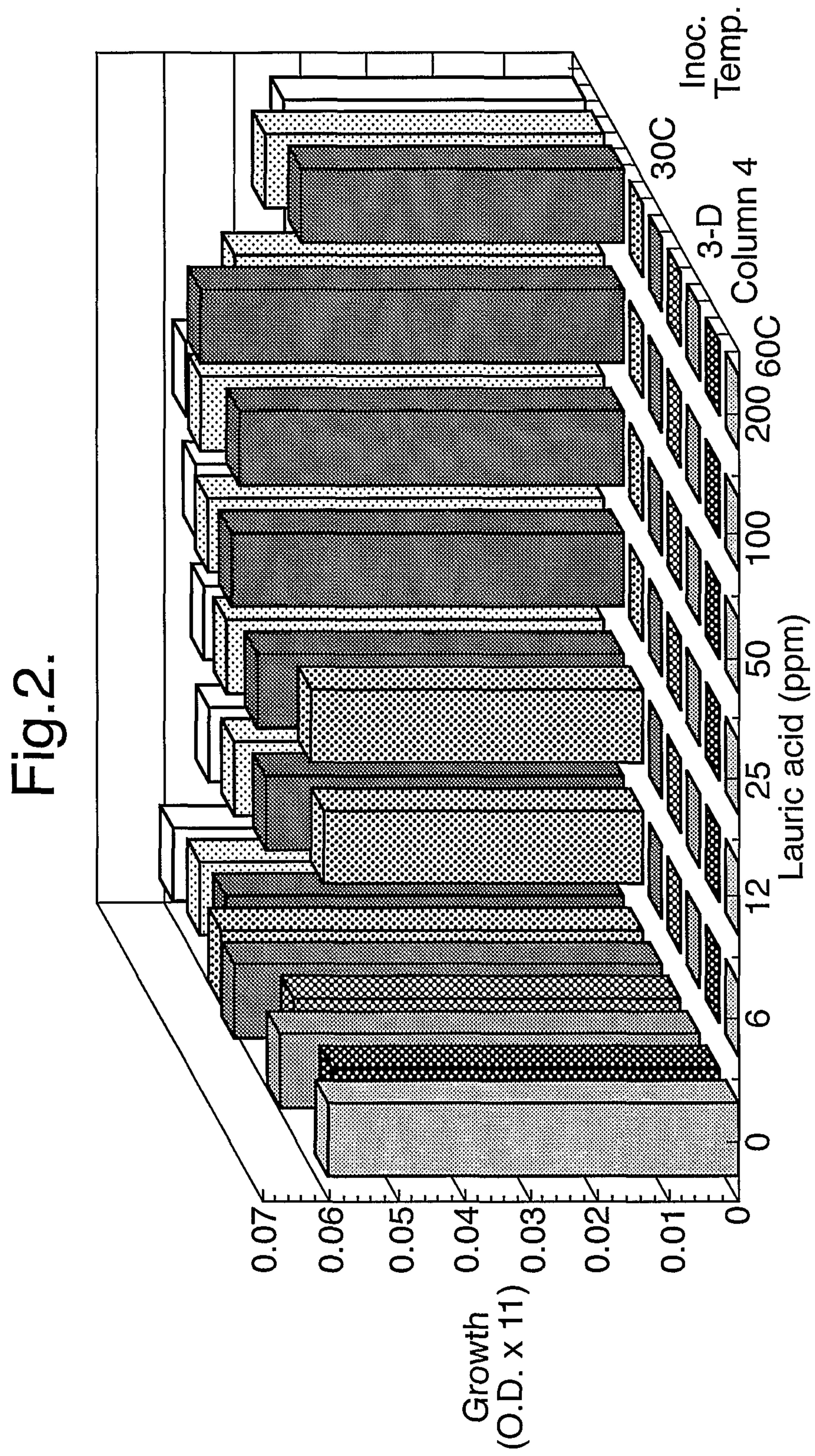
12. A method according to any one of claims 1 to 11,
wherein the pasteurisation adjunct also becomes
20 antibacterial when activated by heat.

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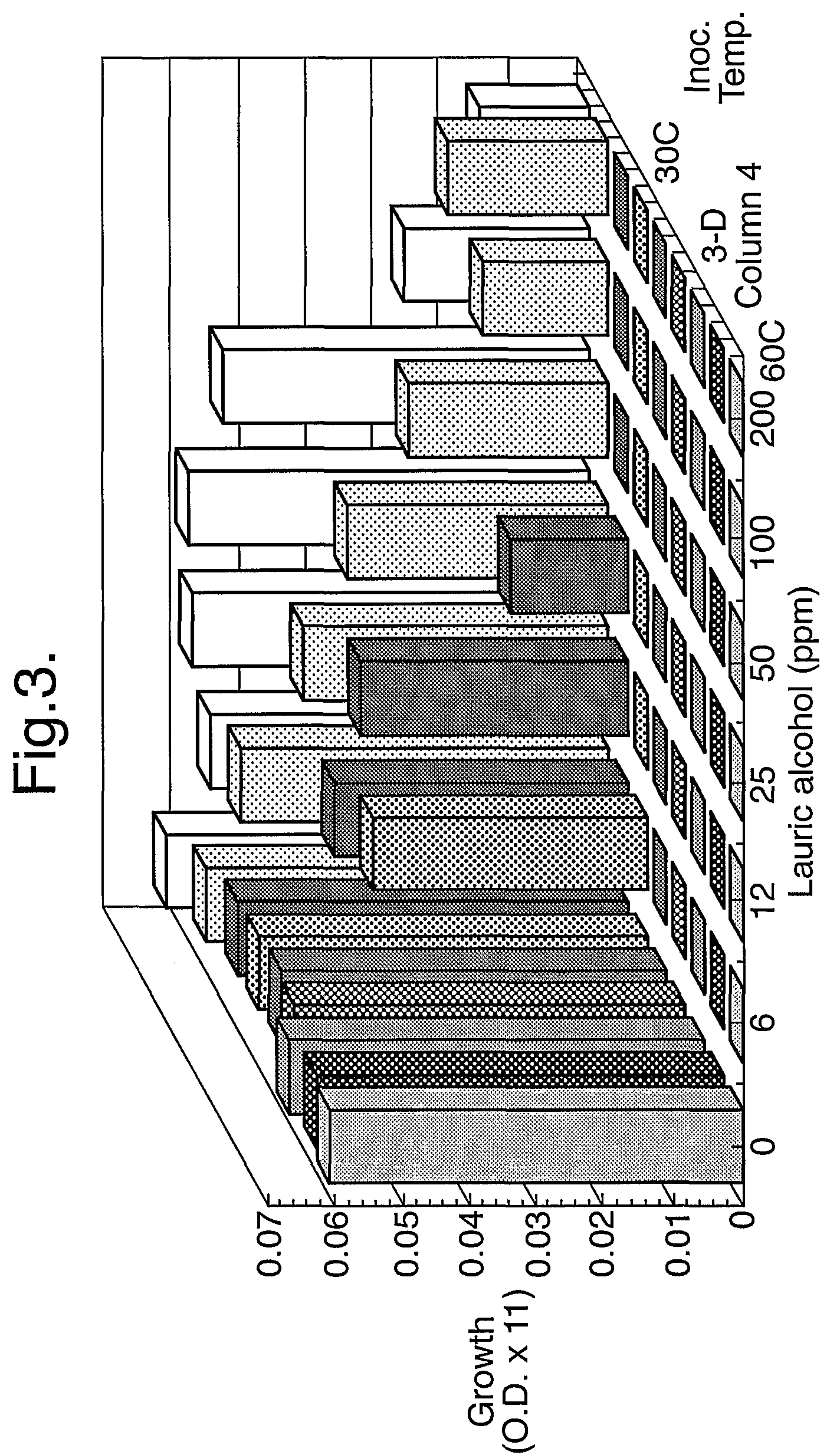
Fig. 1.



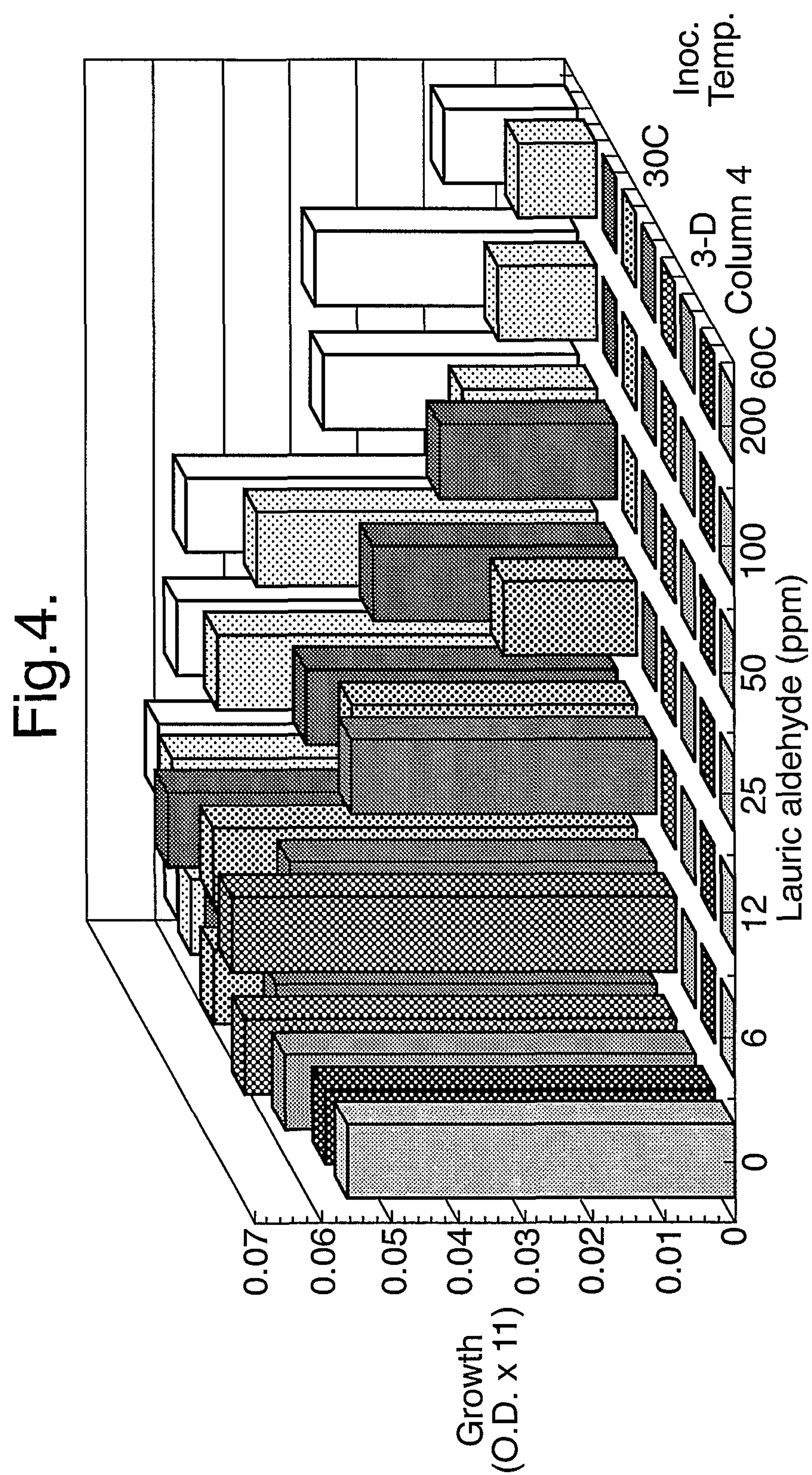
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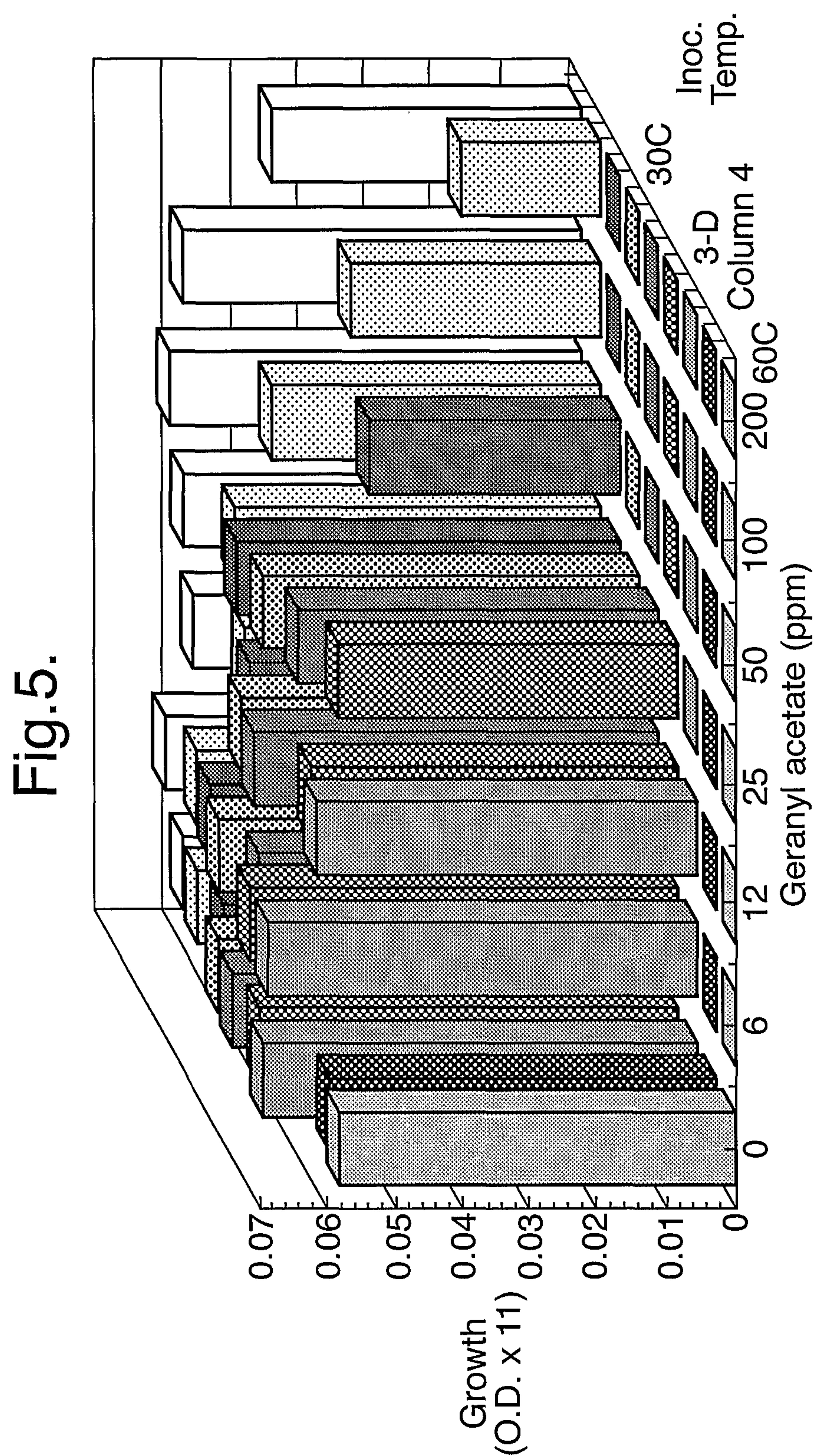
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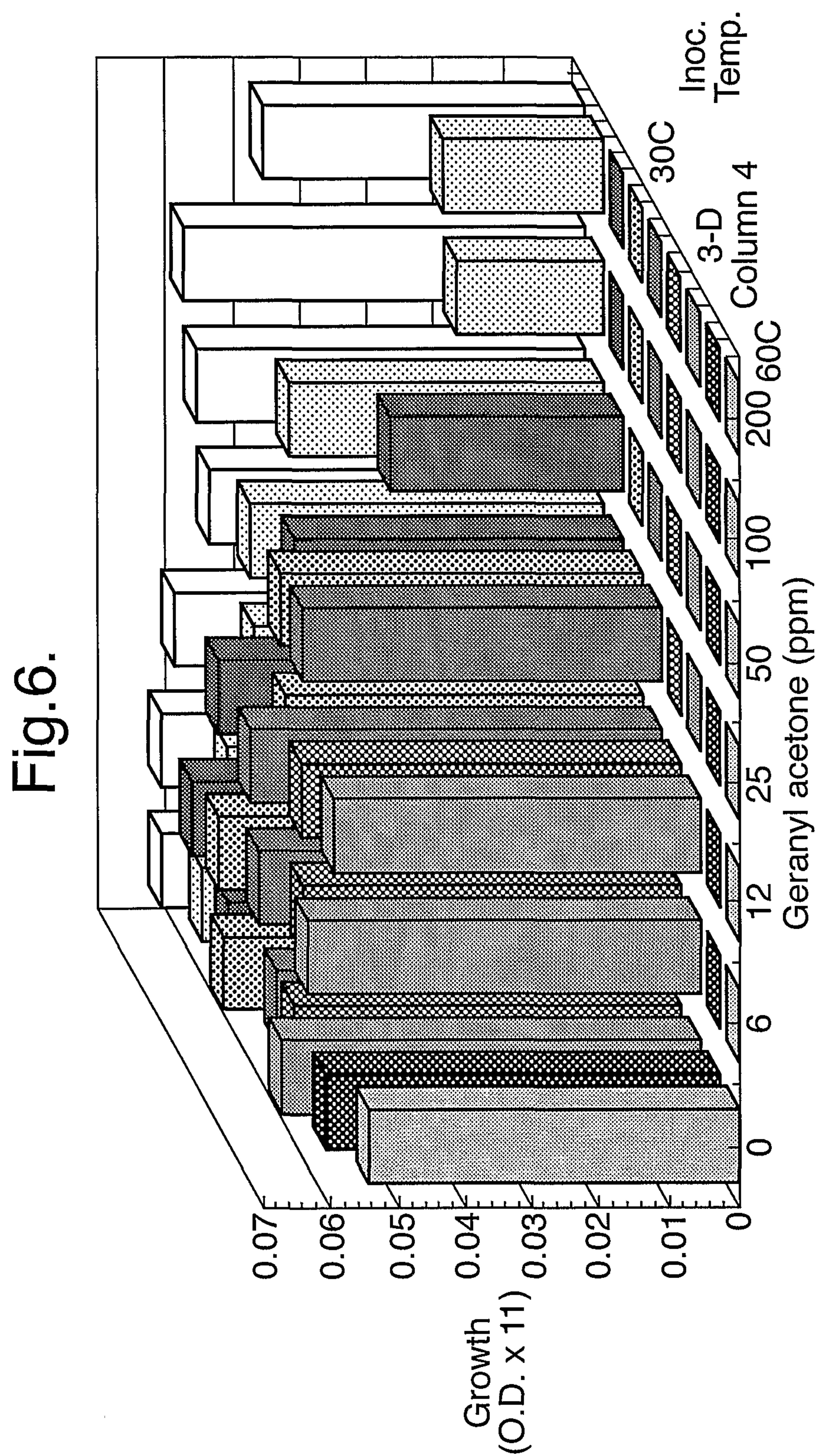
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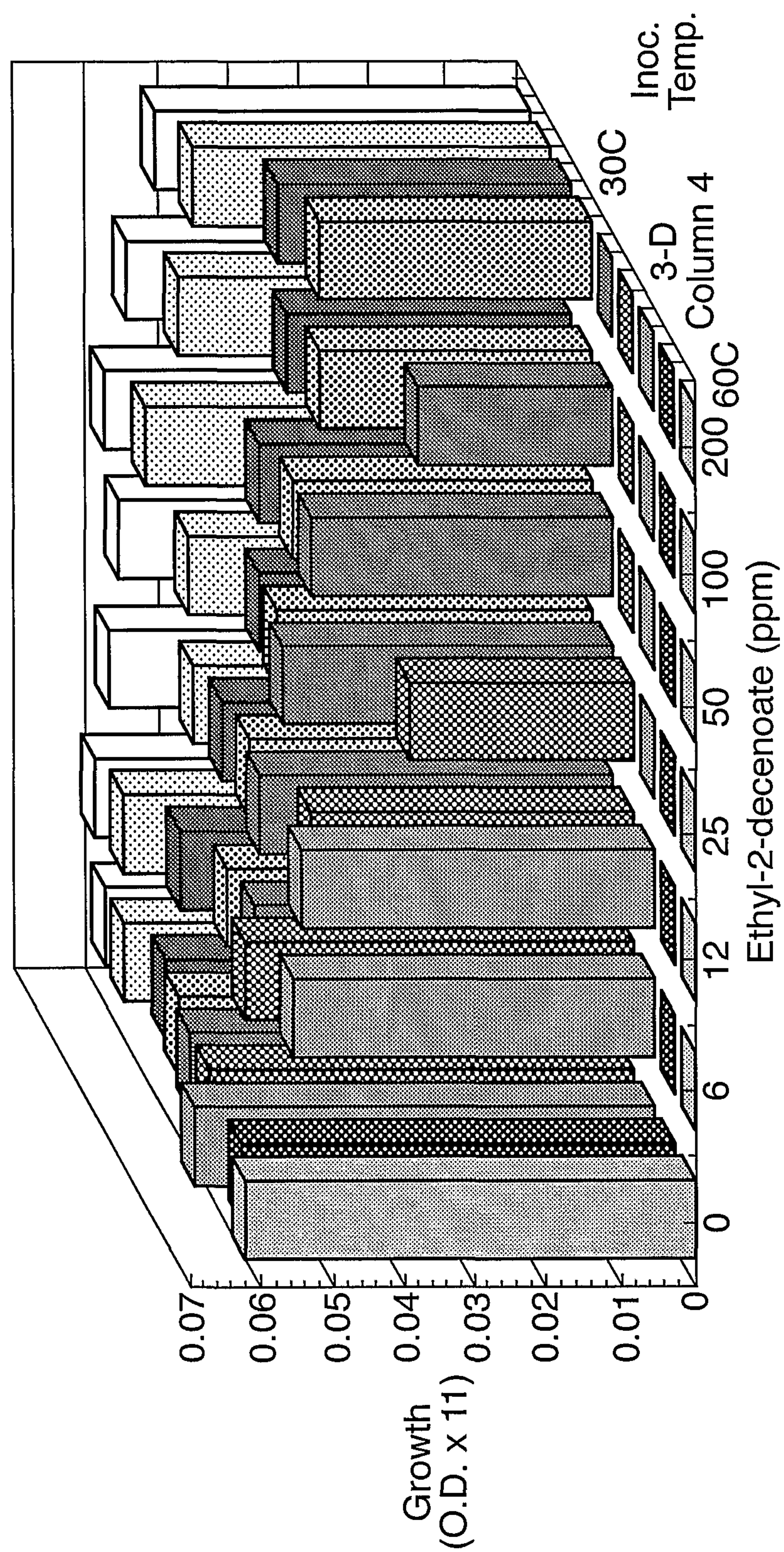


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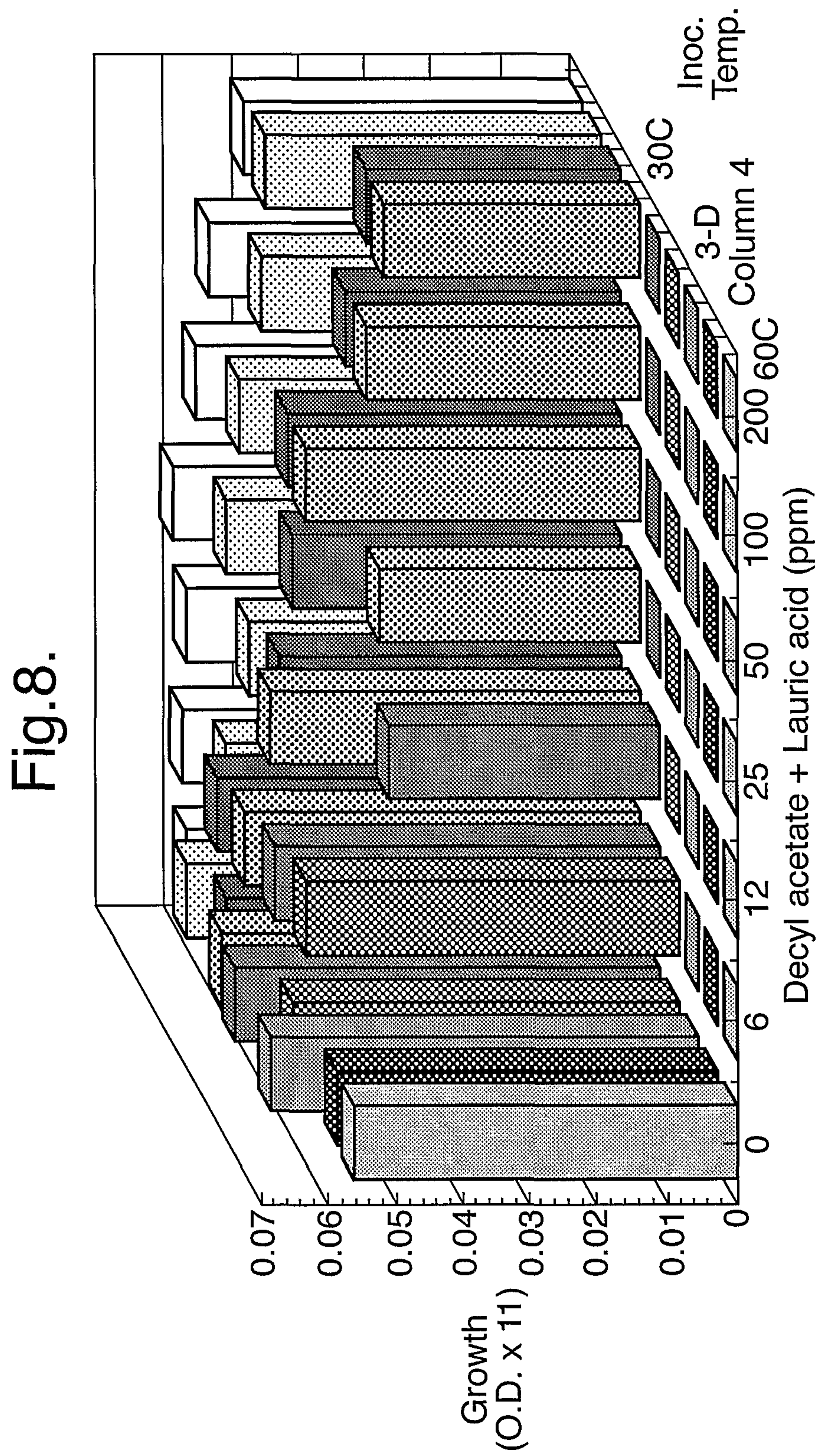


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Fig.7.



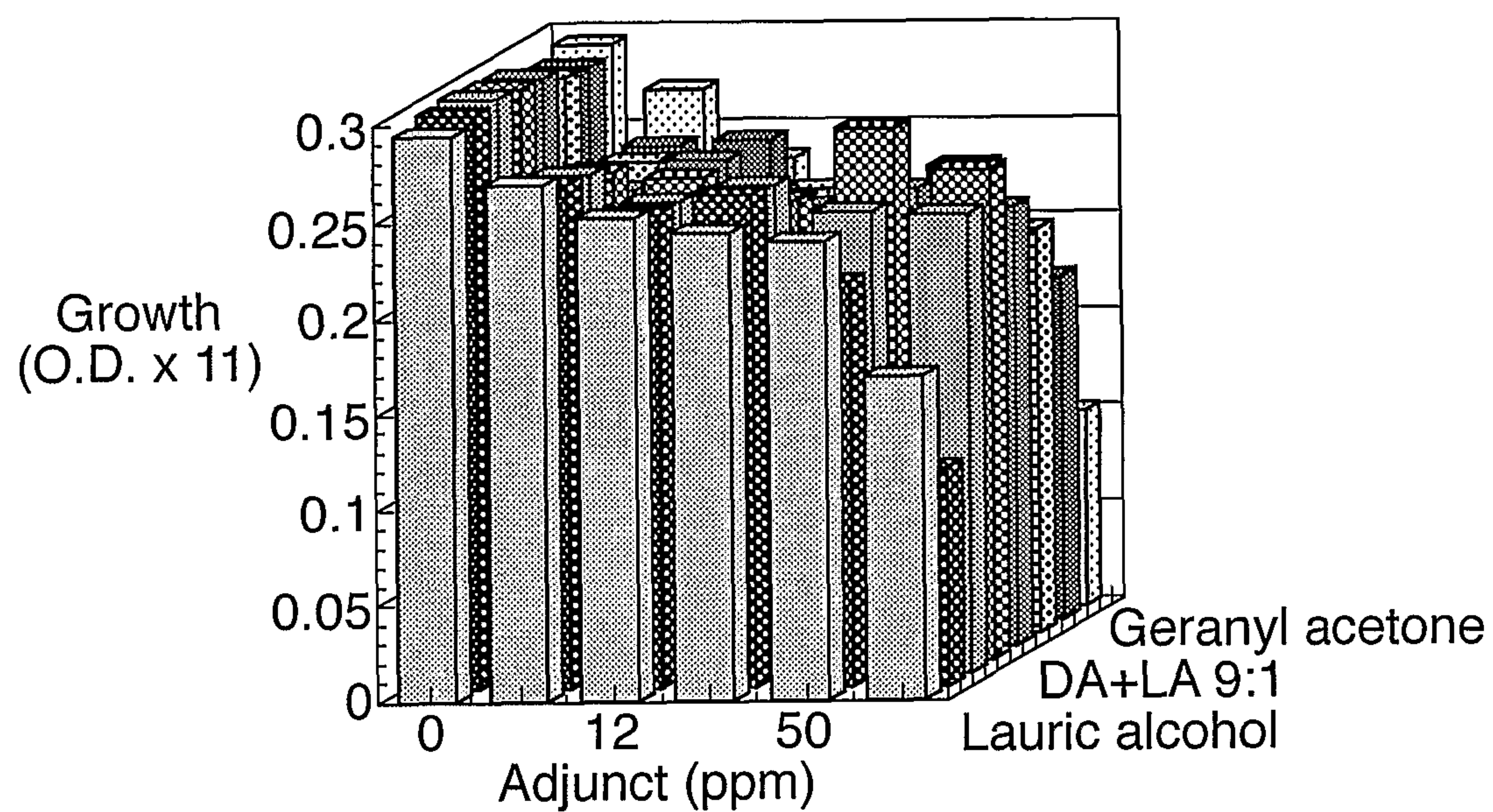
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Fig.9.

20C



50C

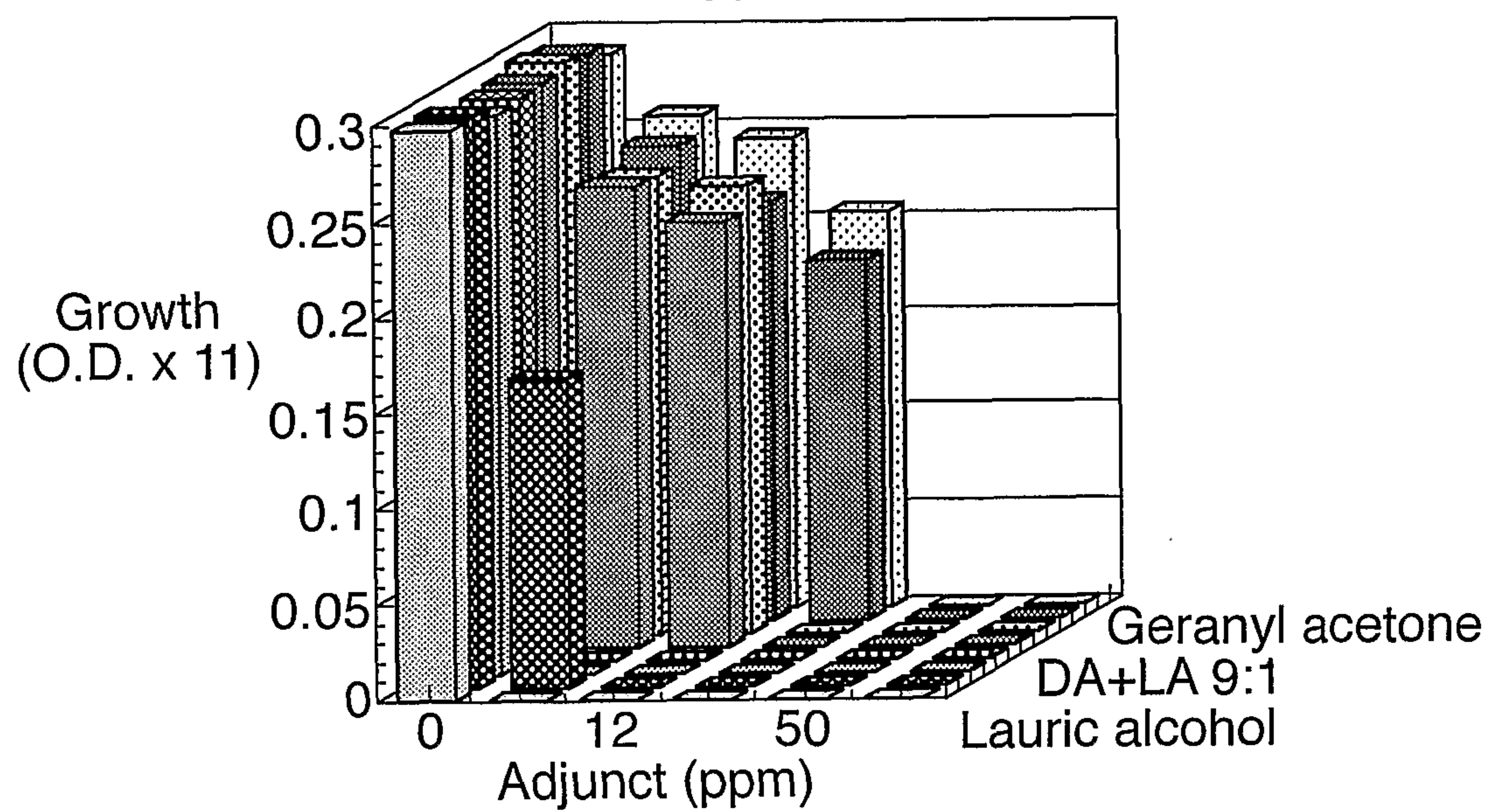
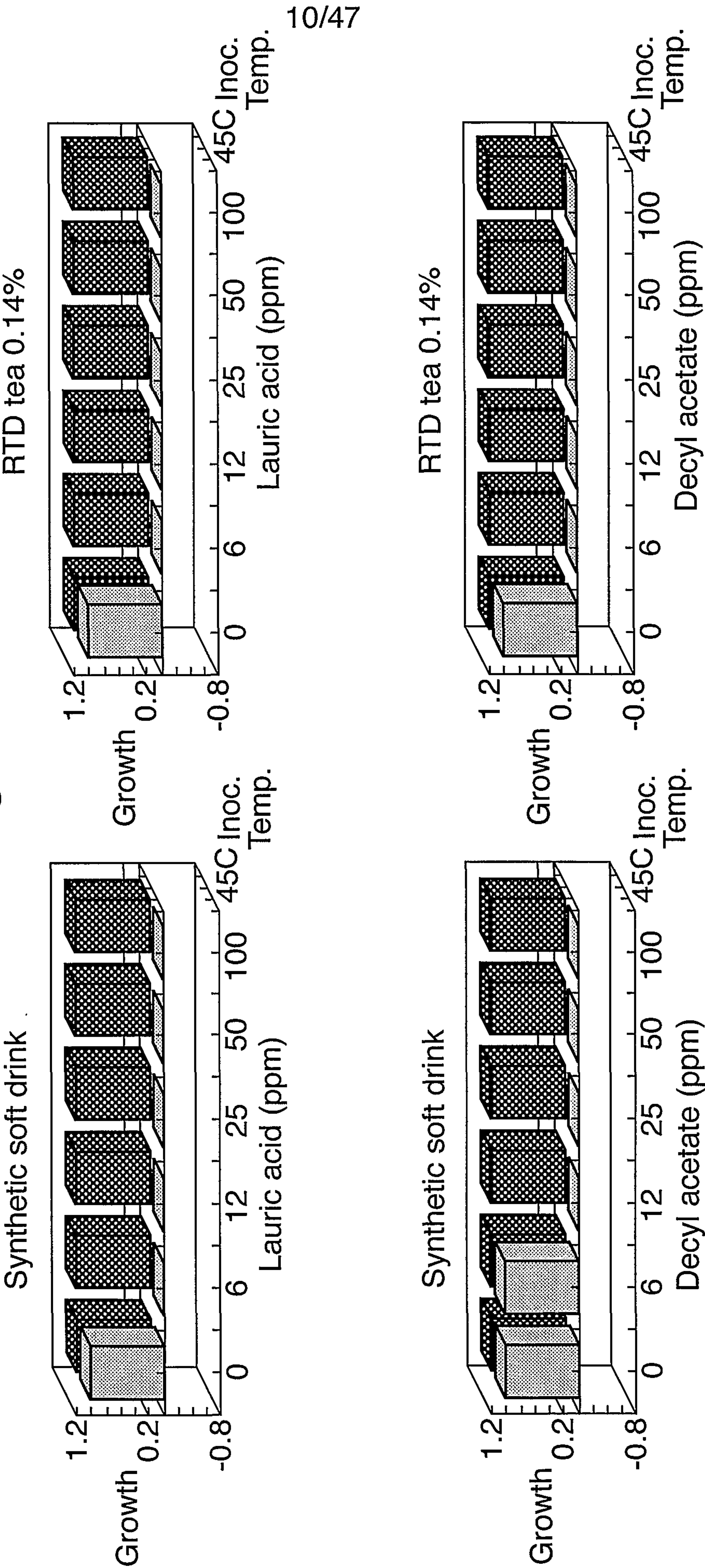
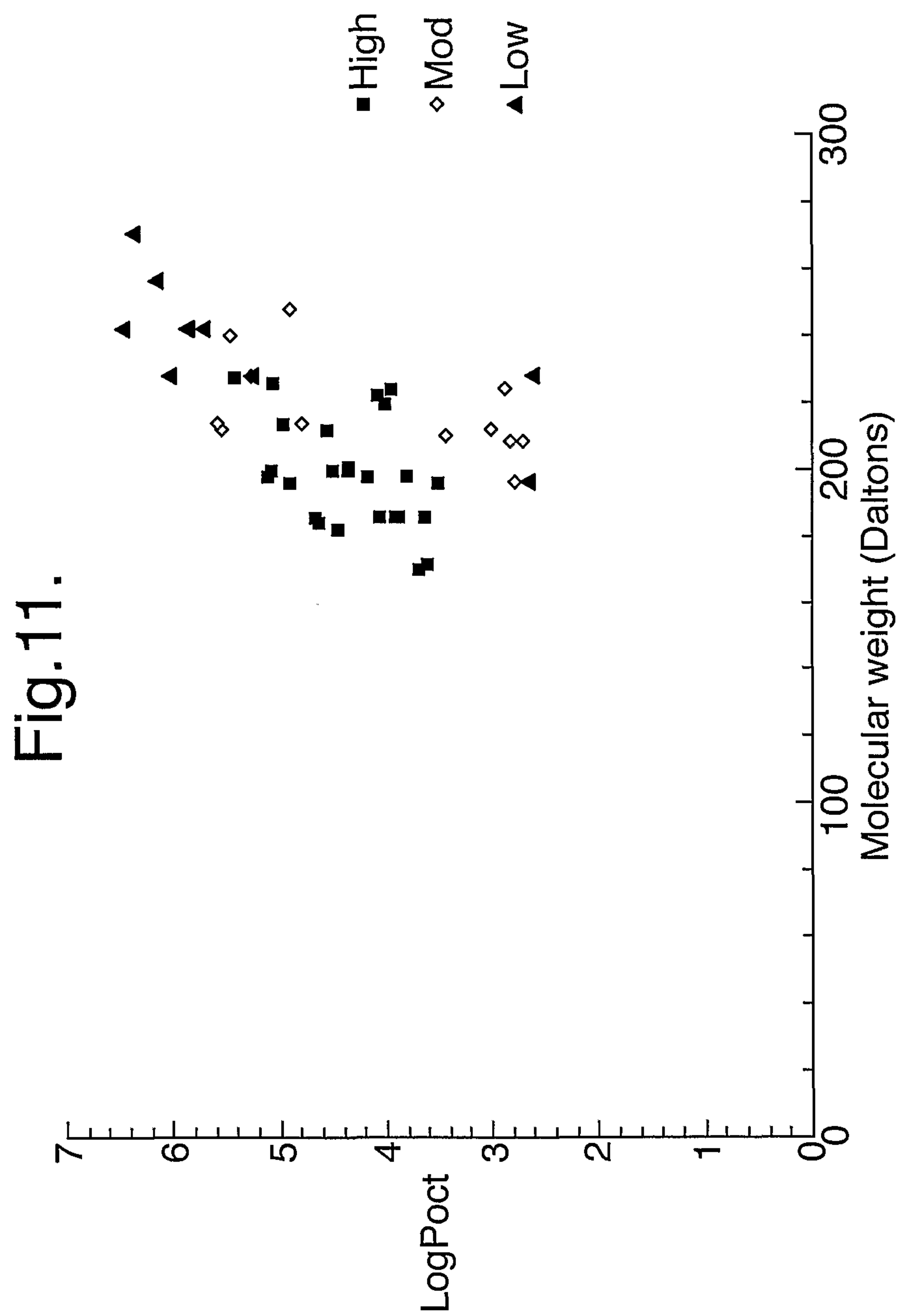


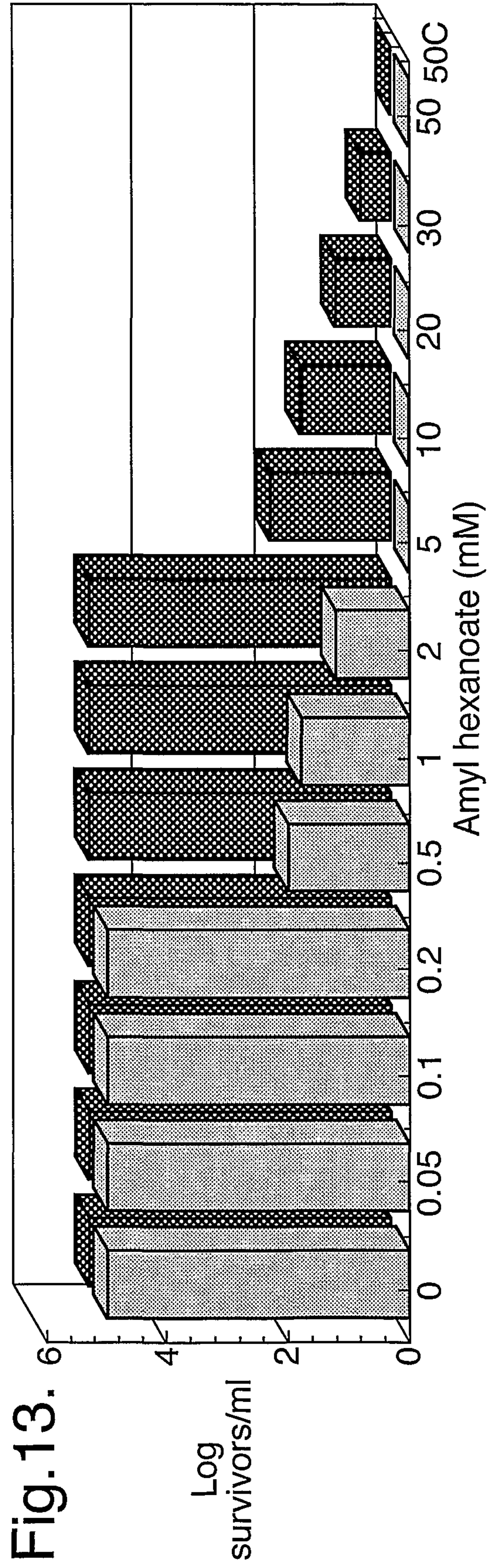
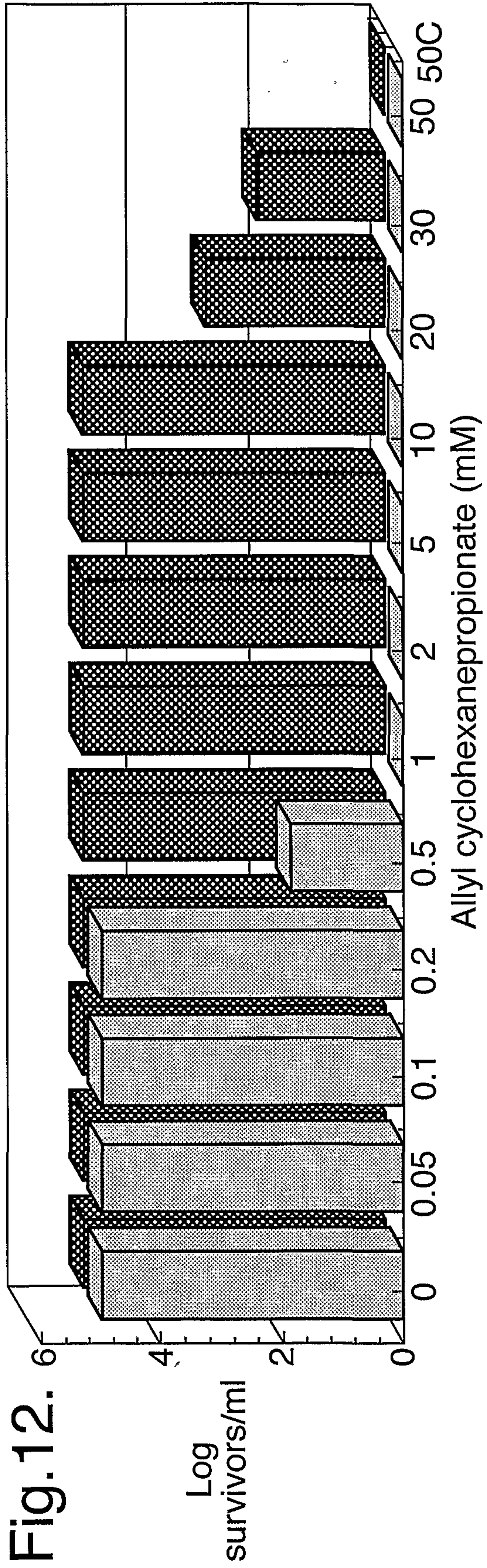
Fig.10.

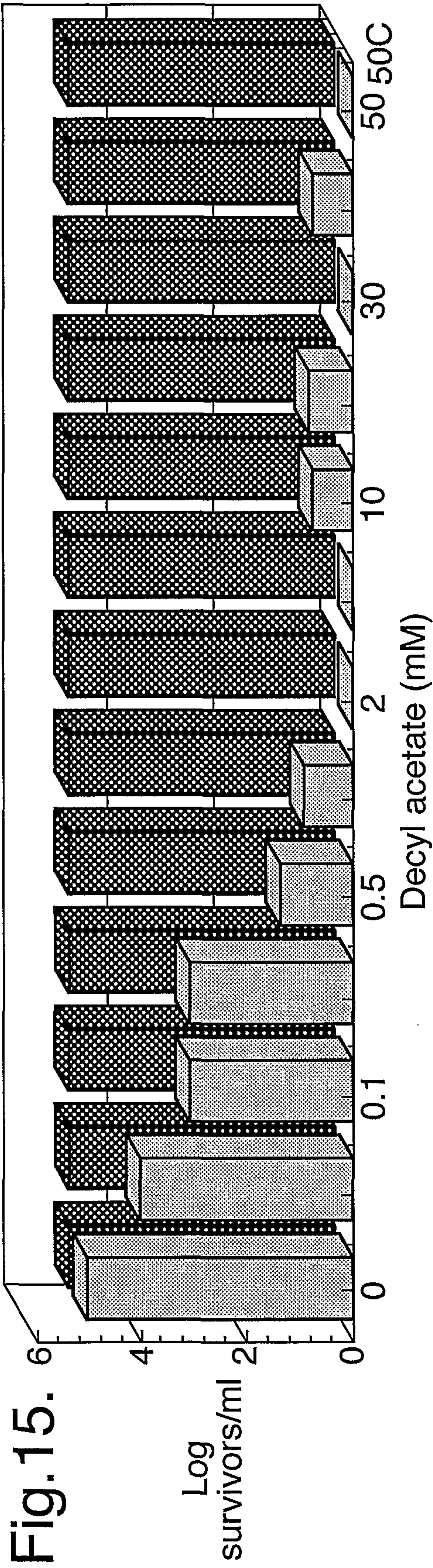
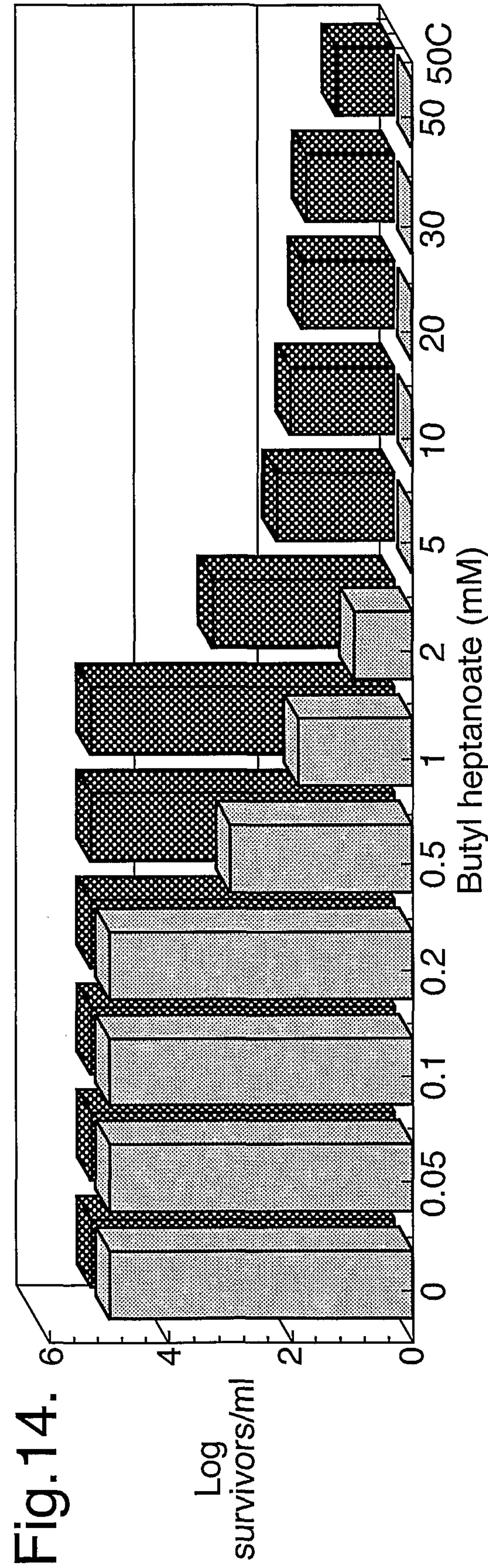


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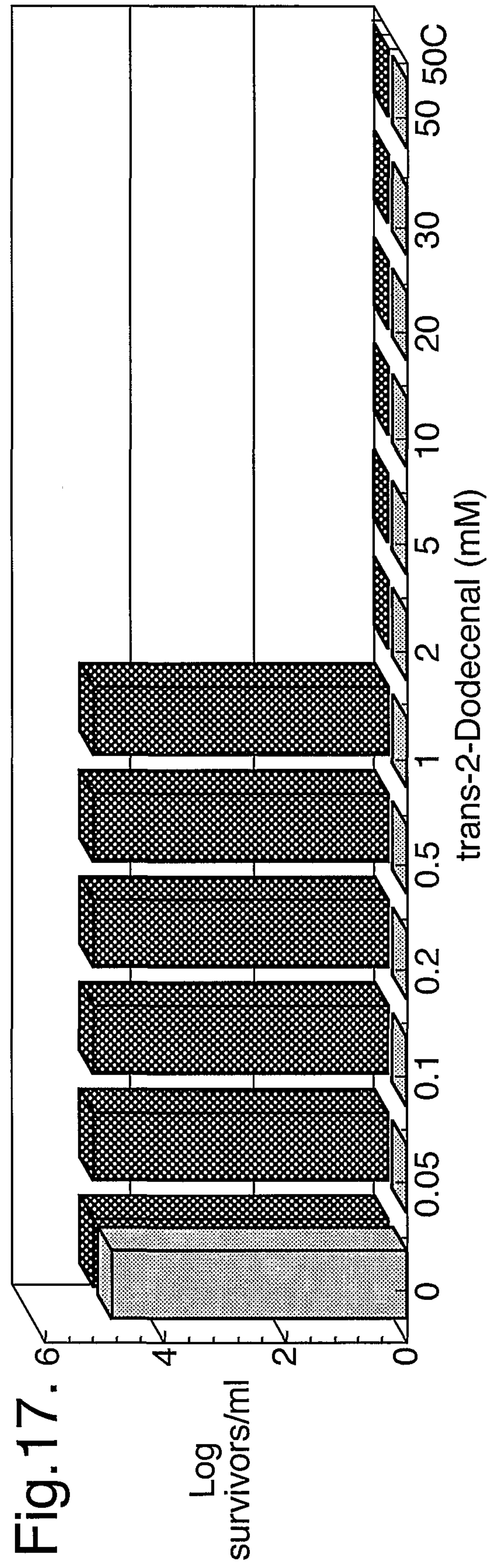
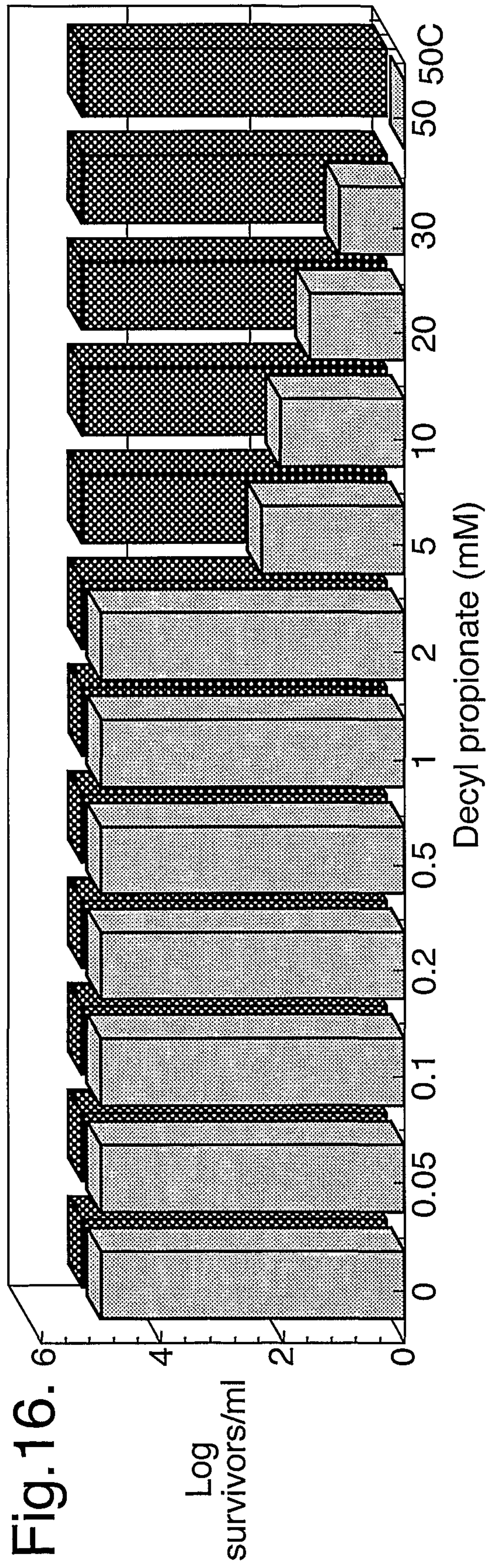


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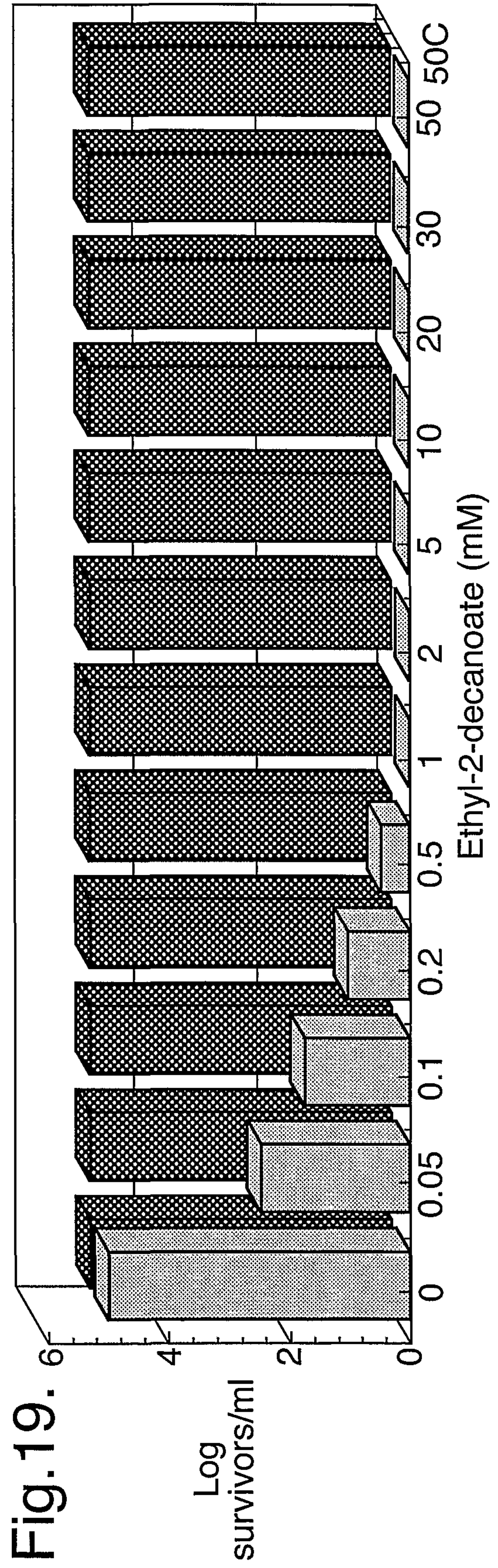
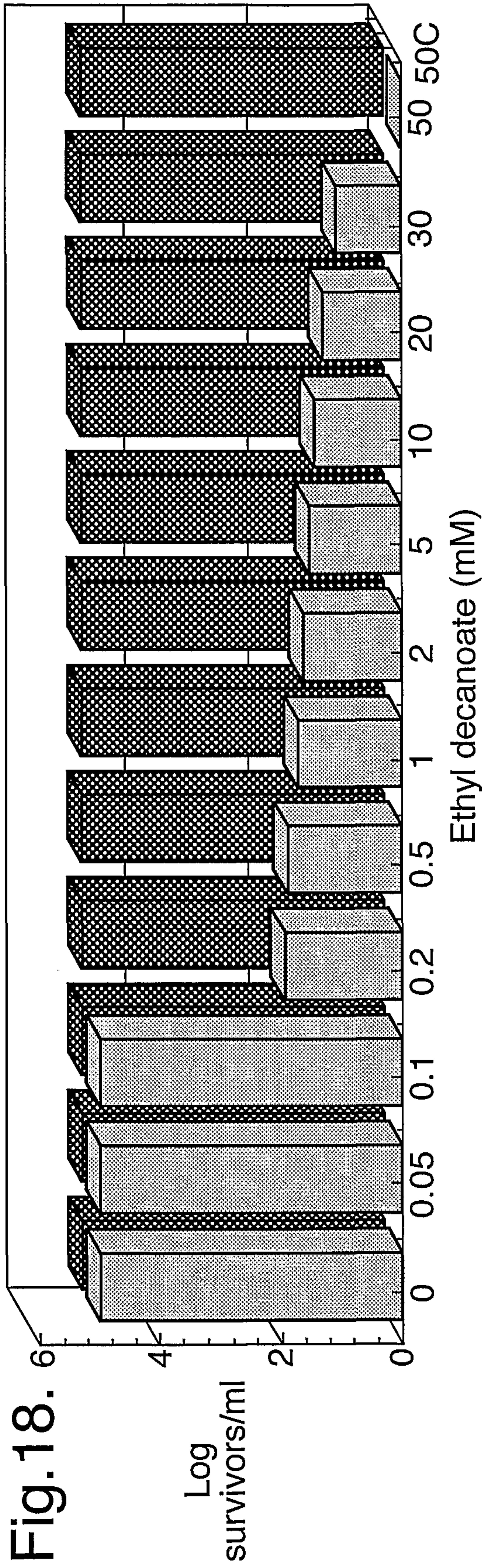




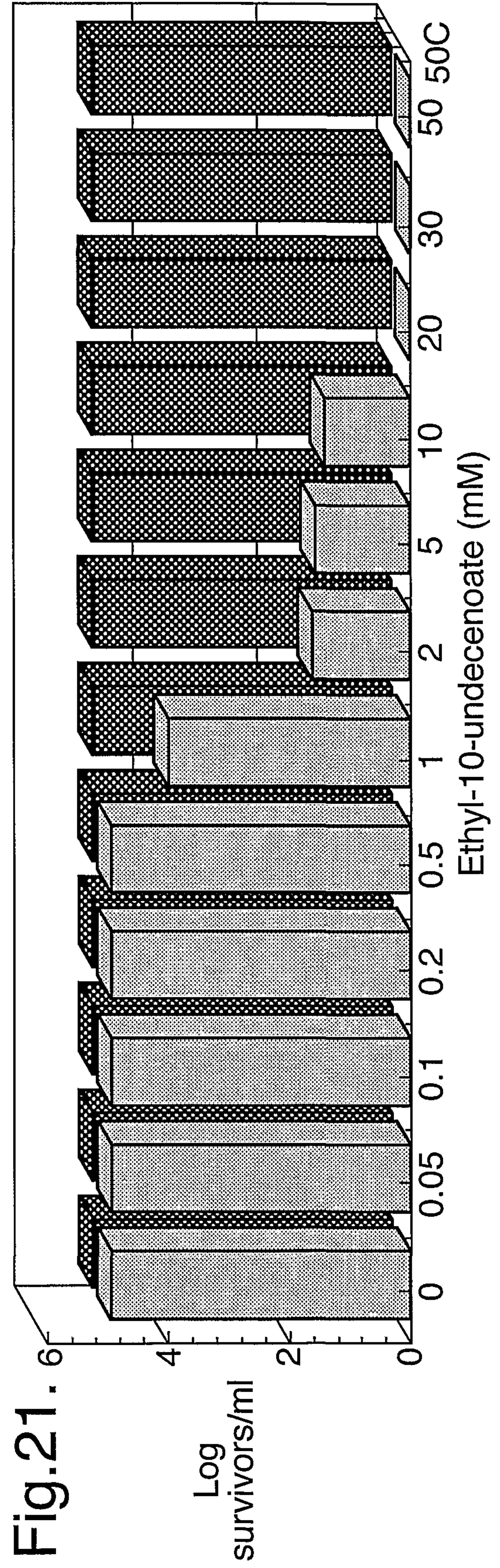
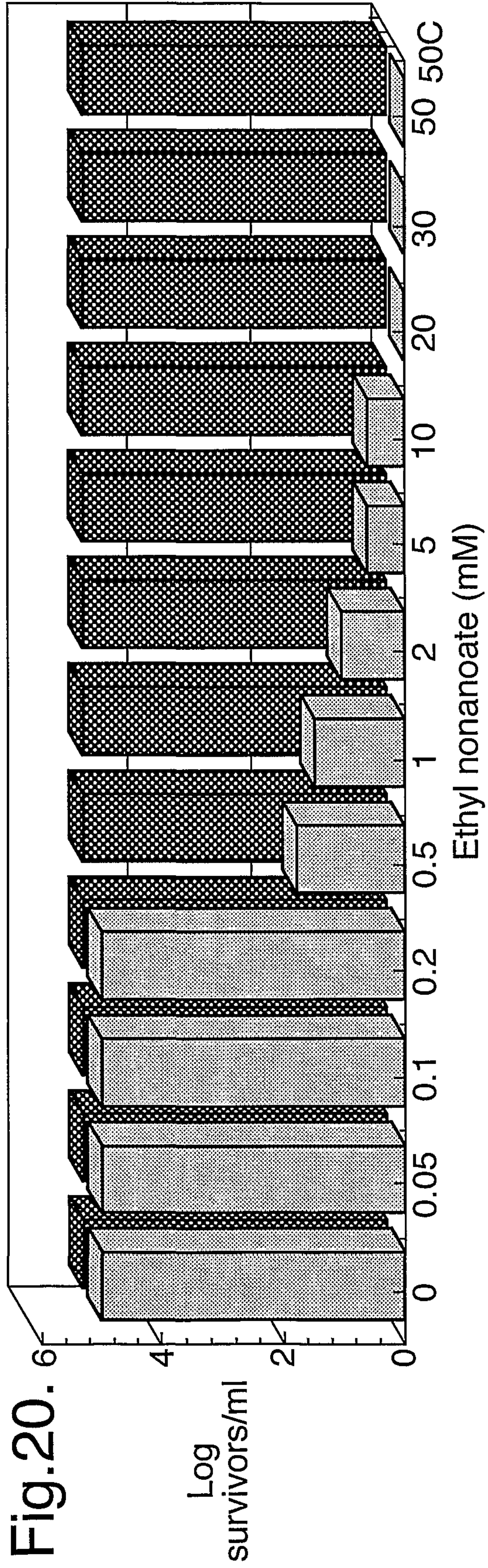
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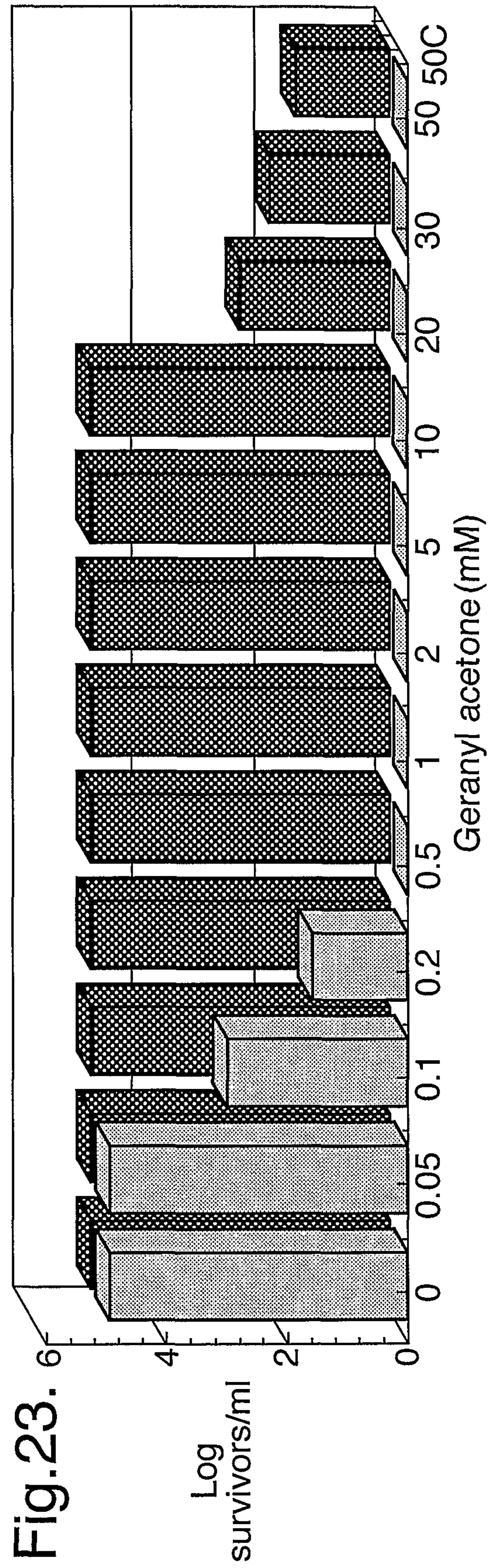
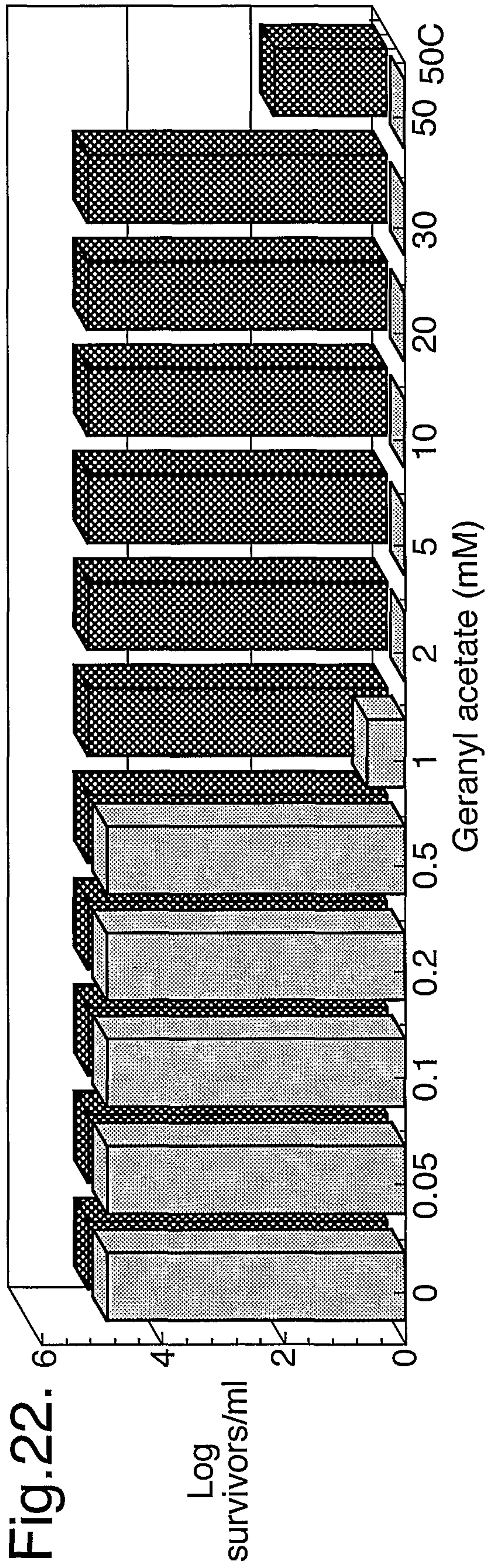
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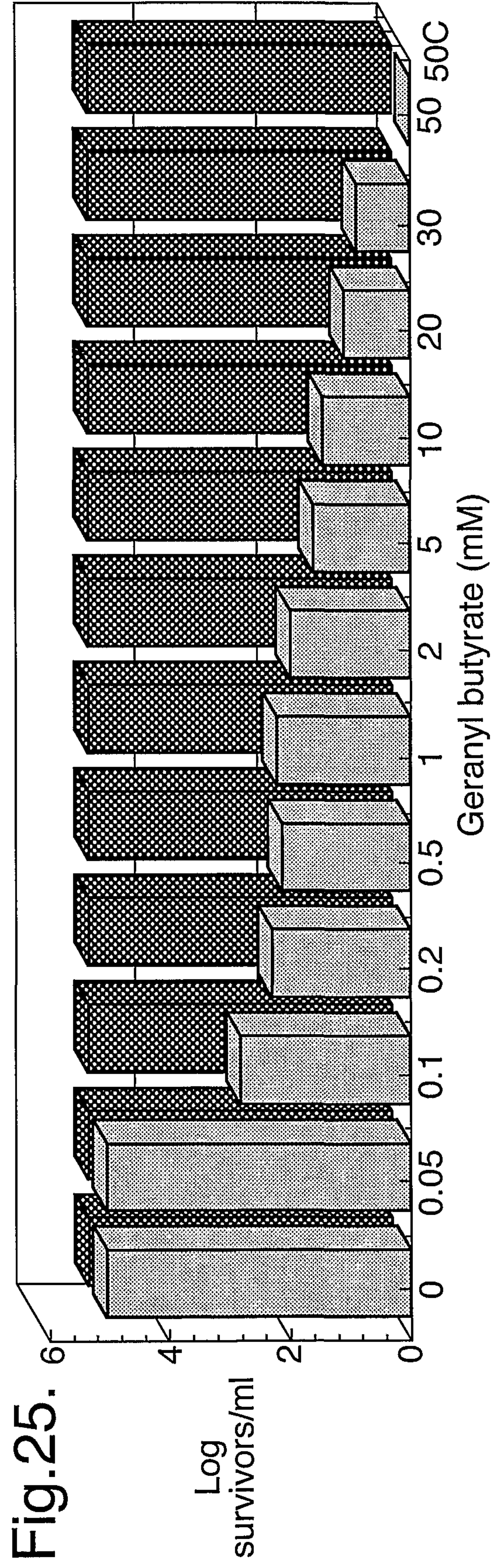
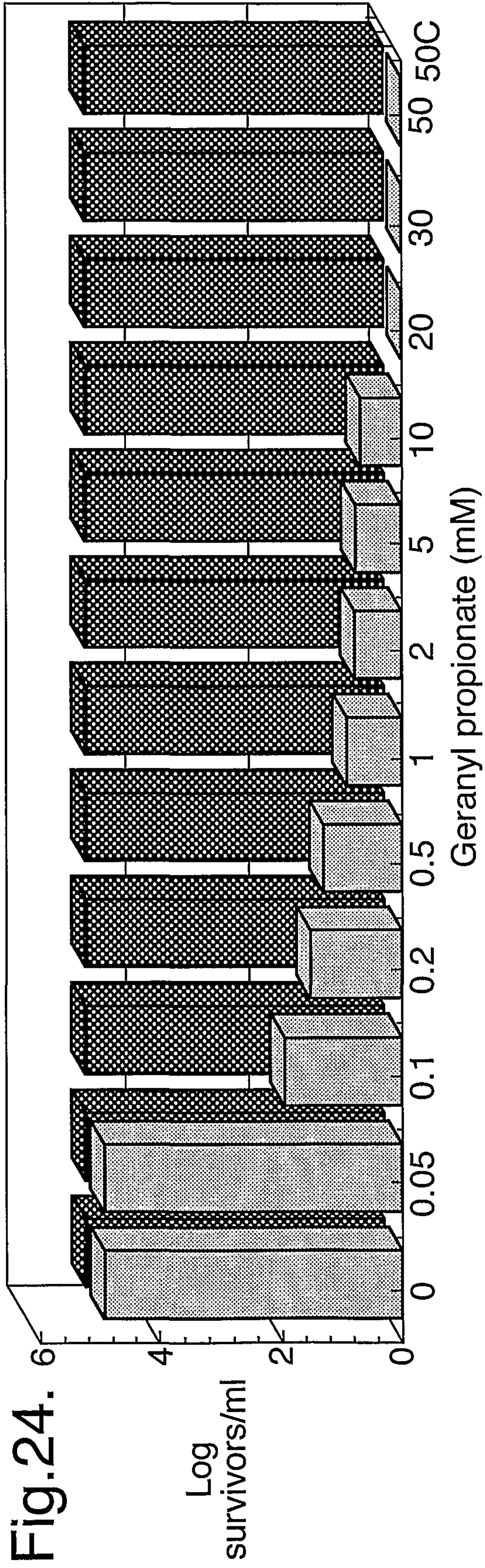
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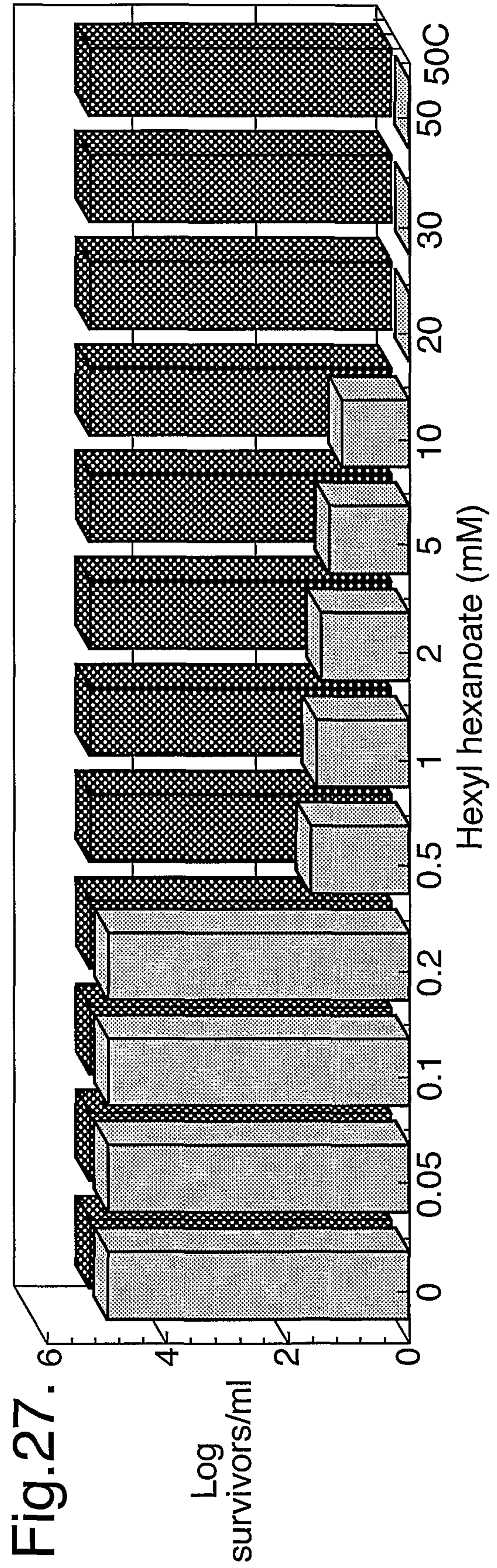
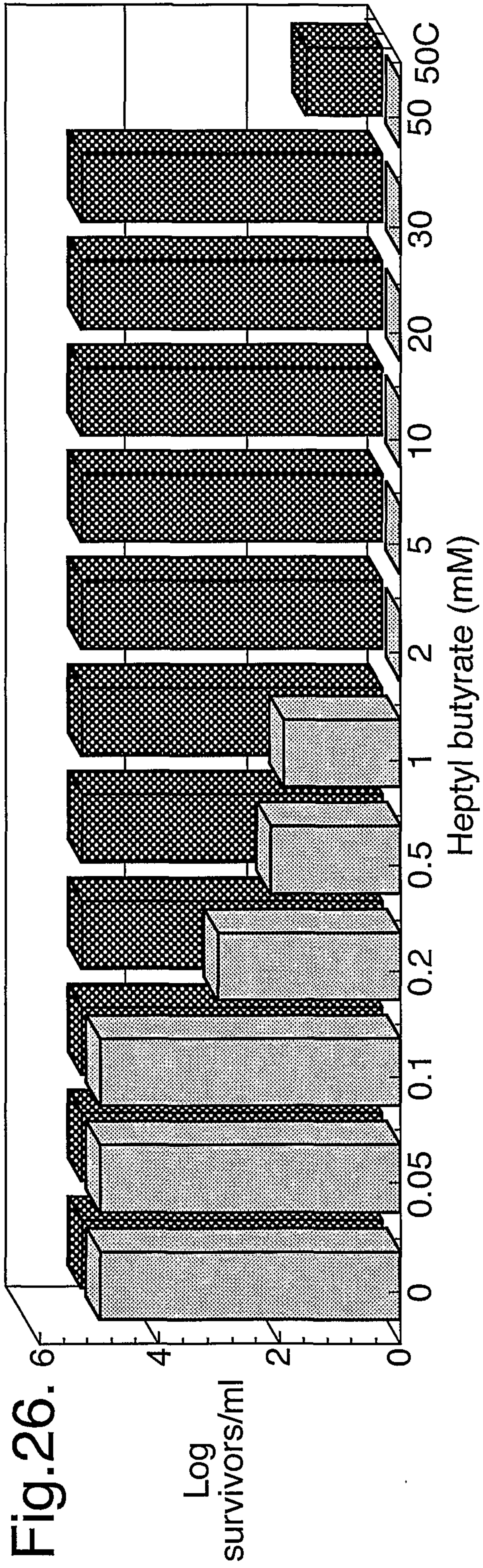
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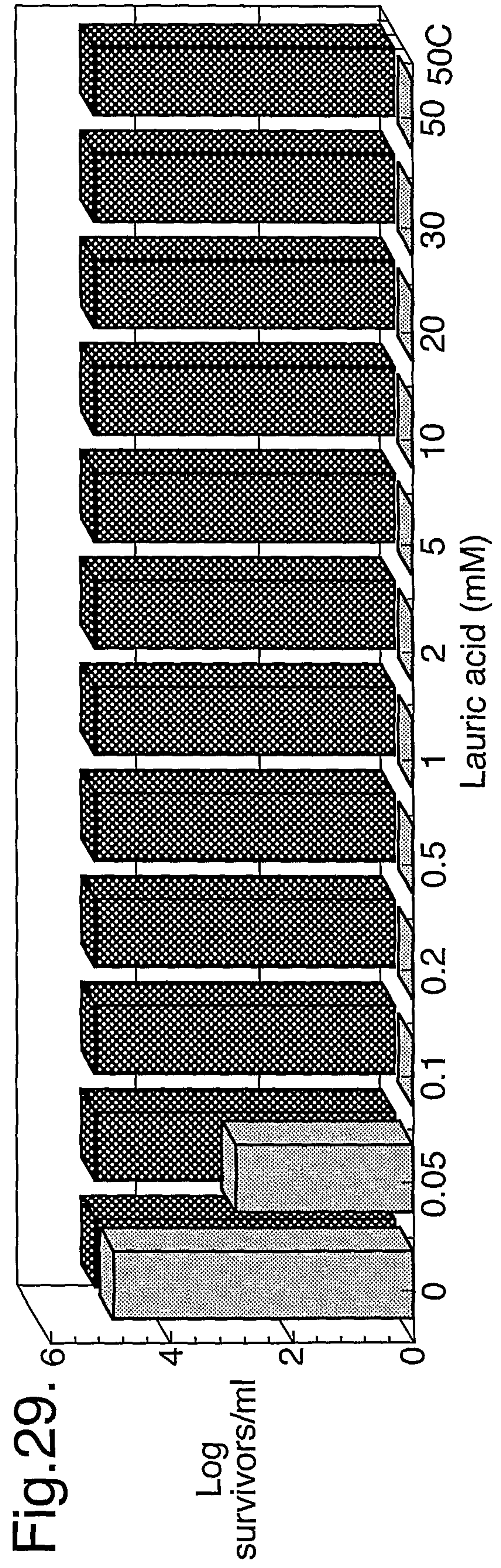
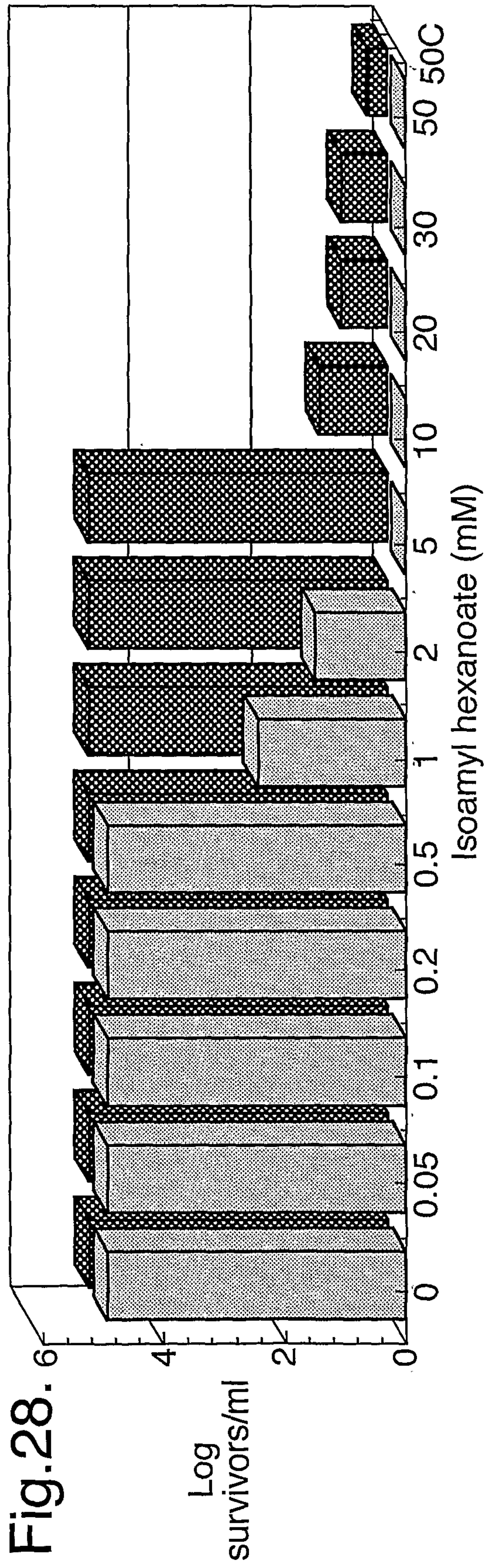
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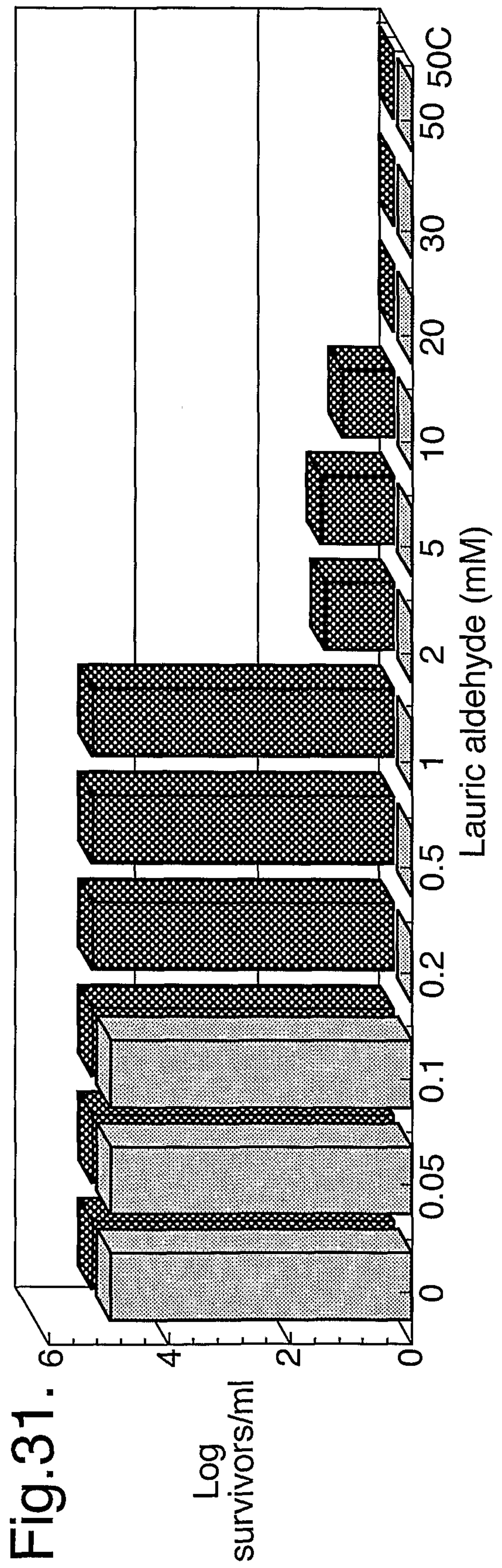
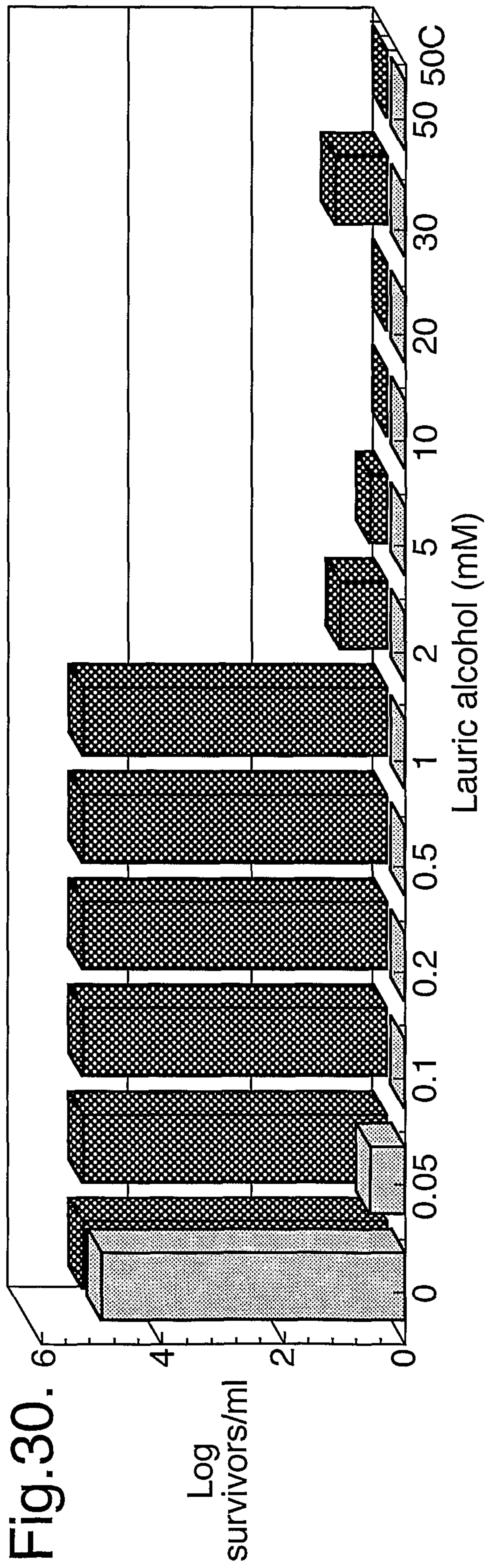
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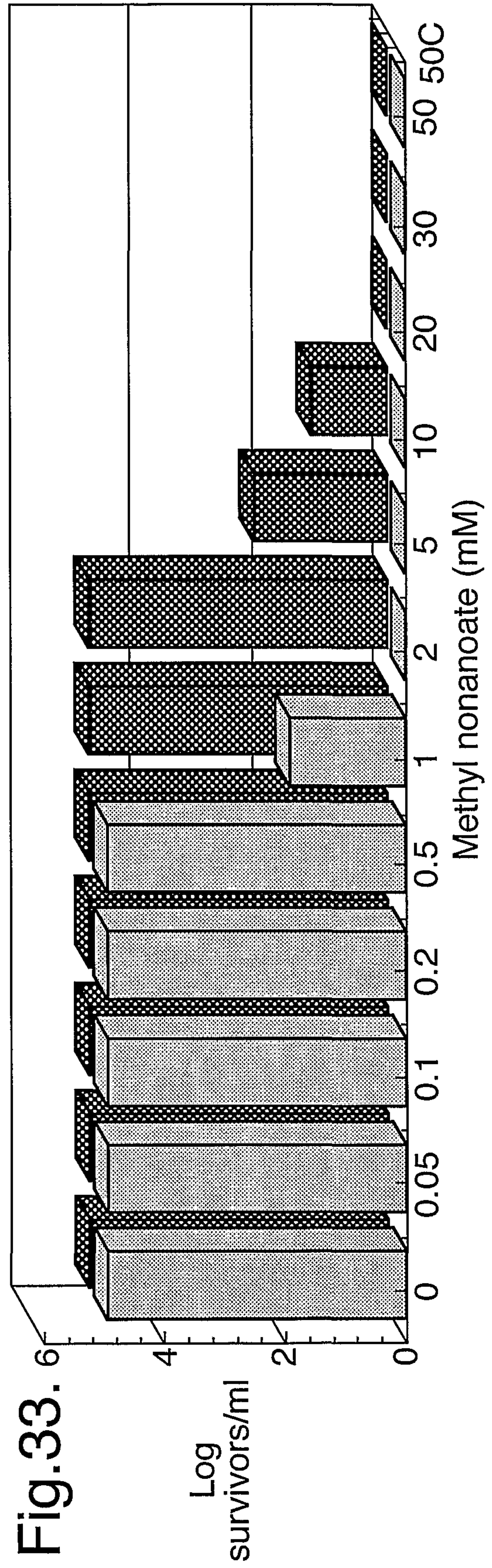
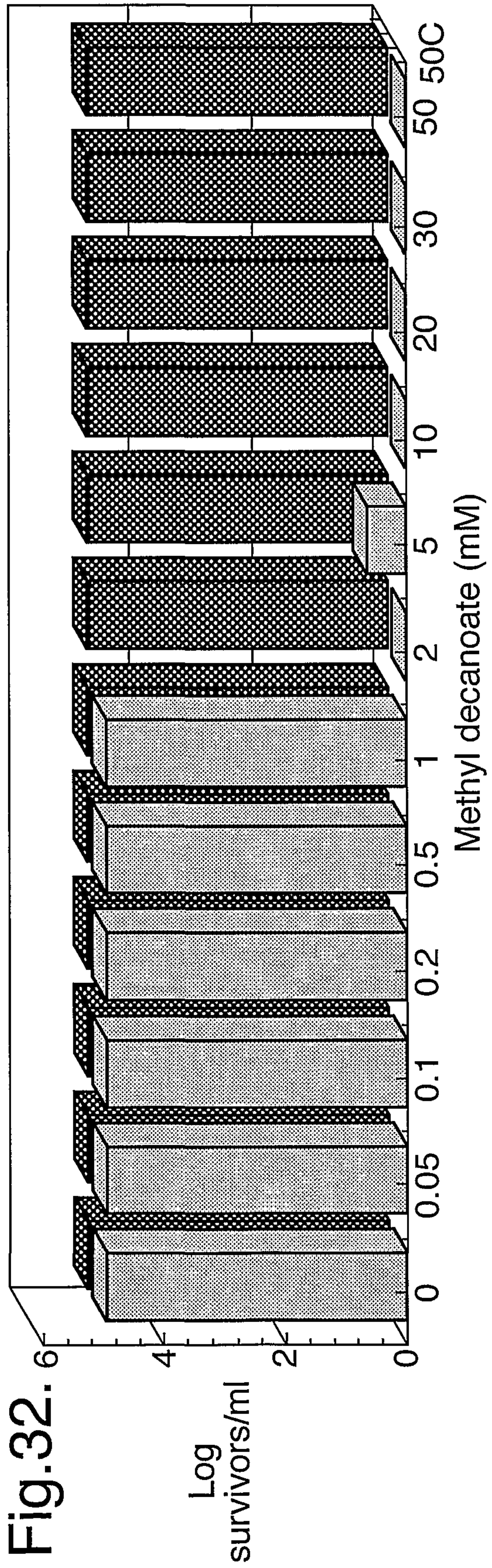
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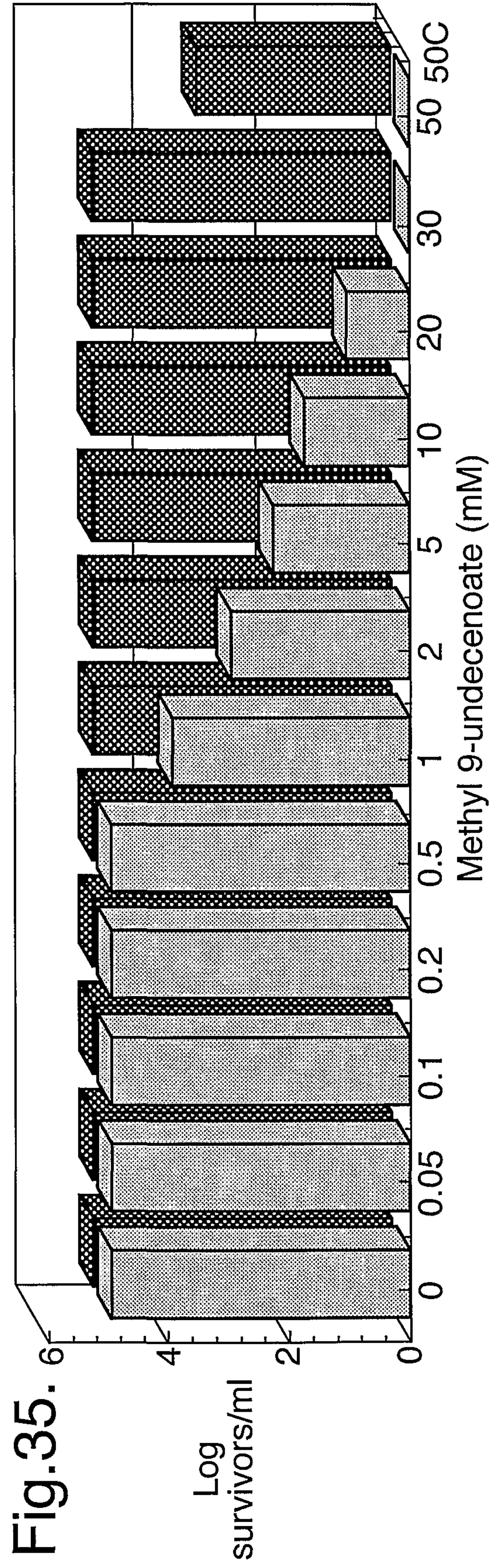
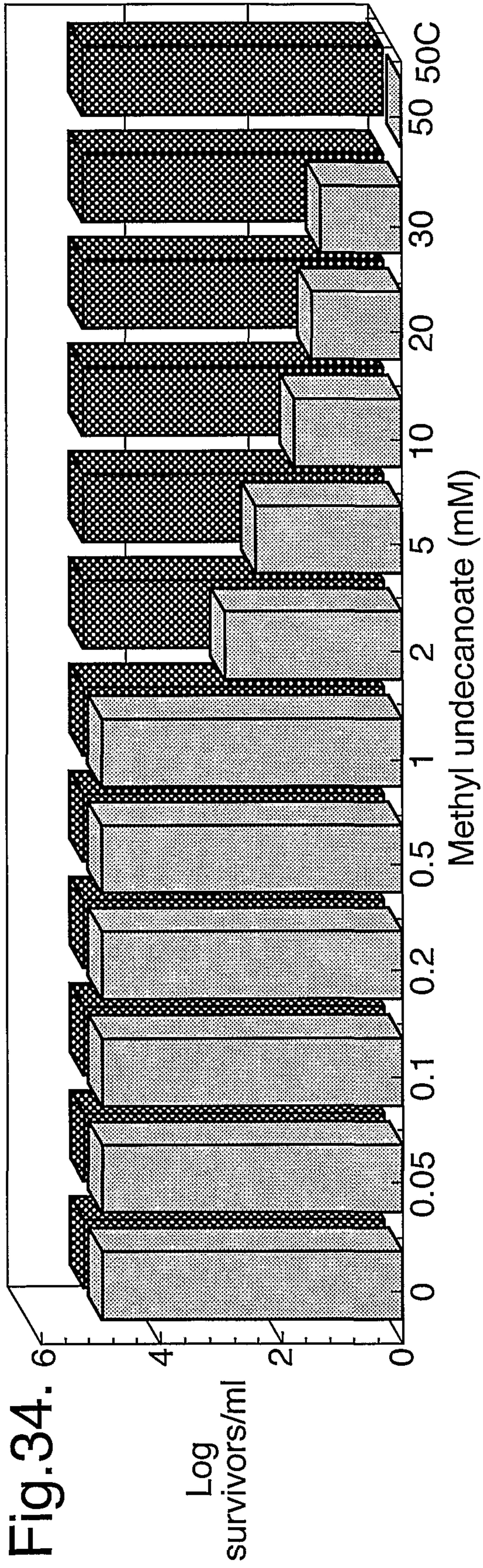
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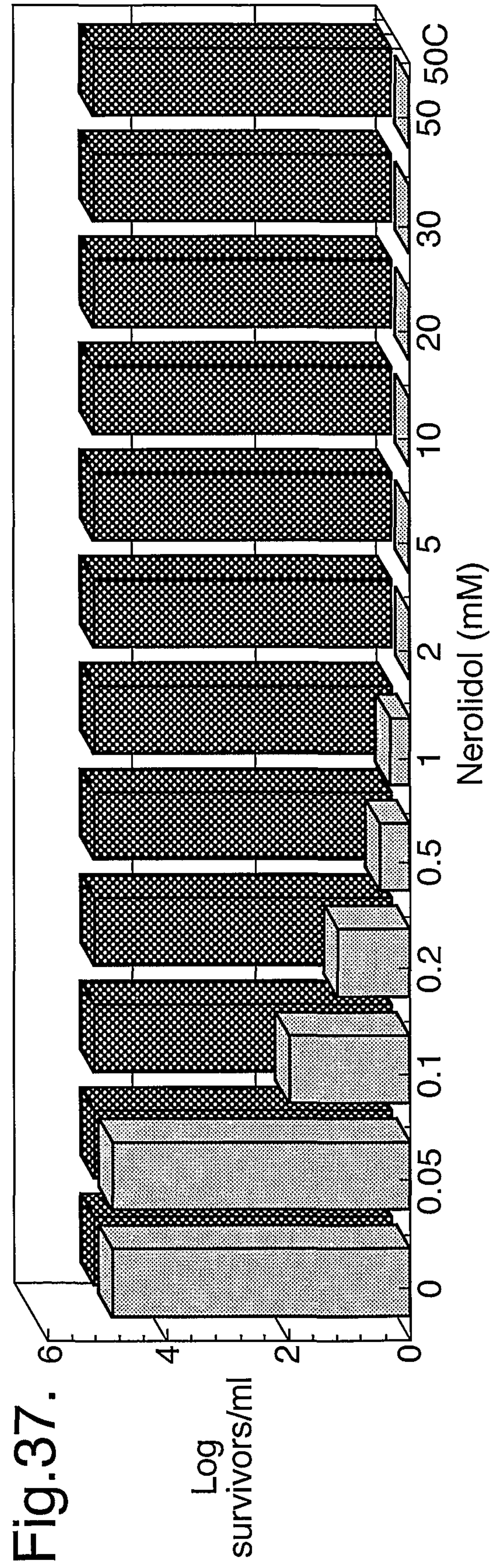
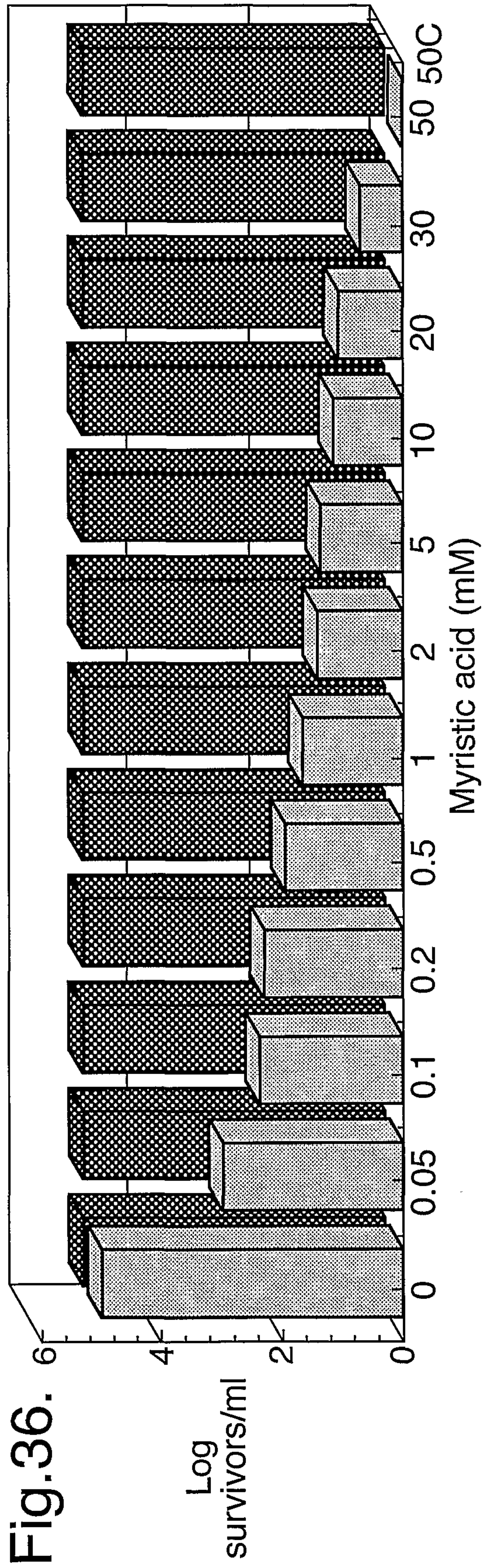
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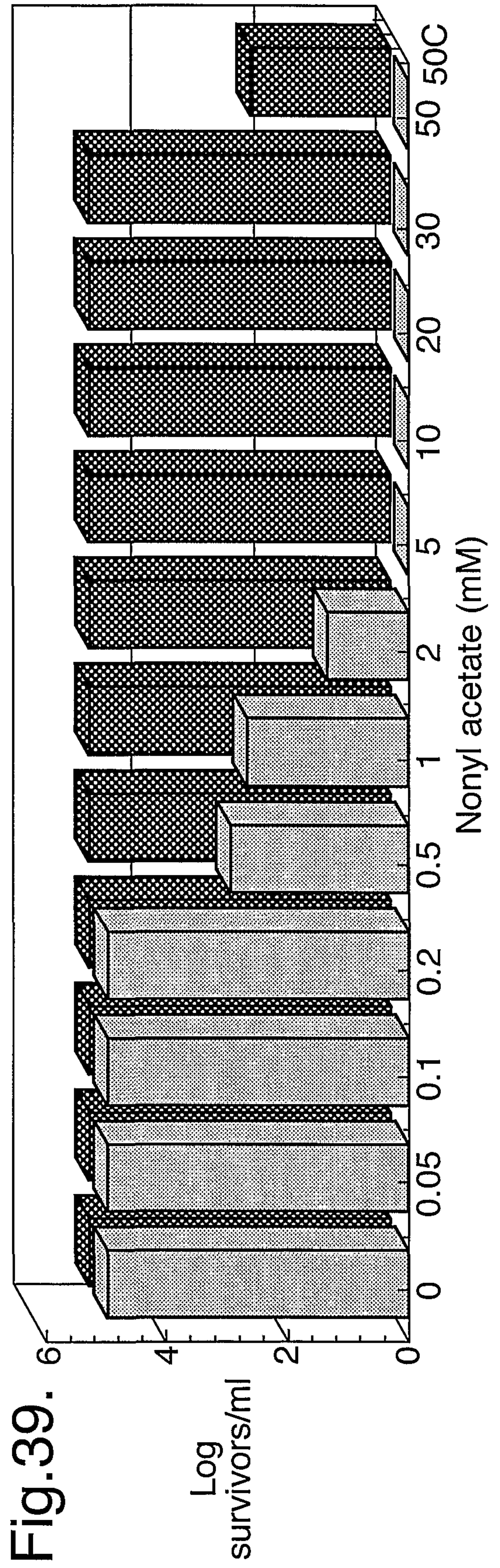
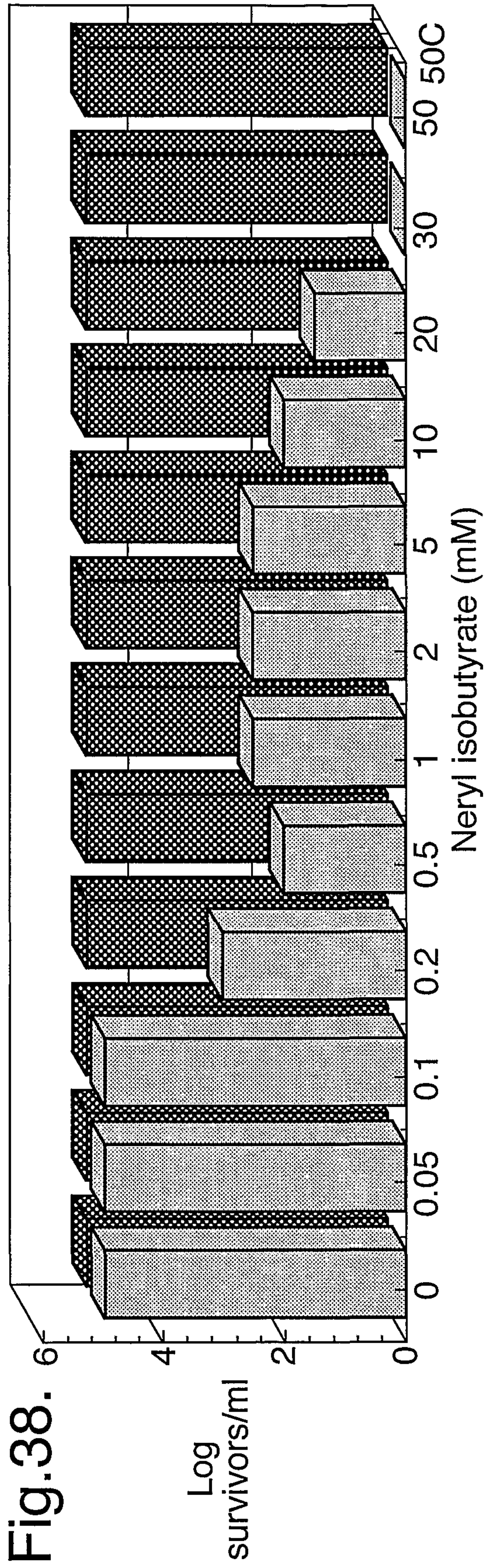
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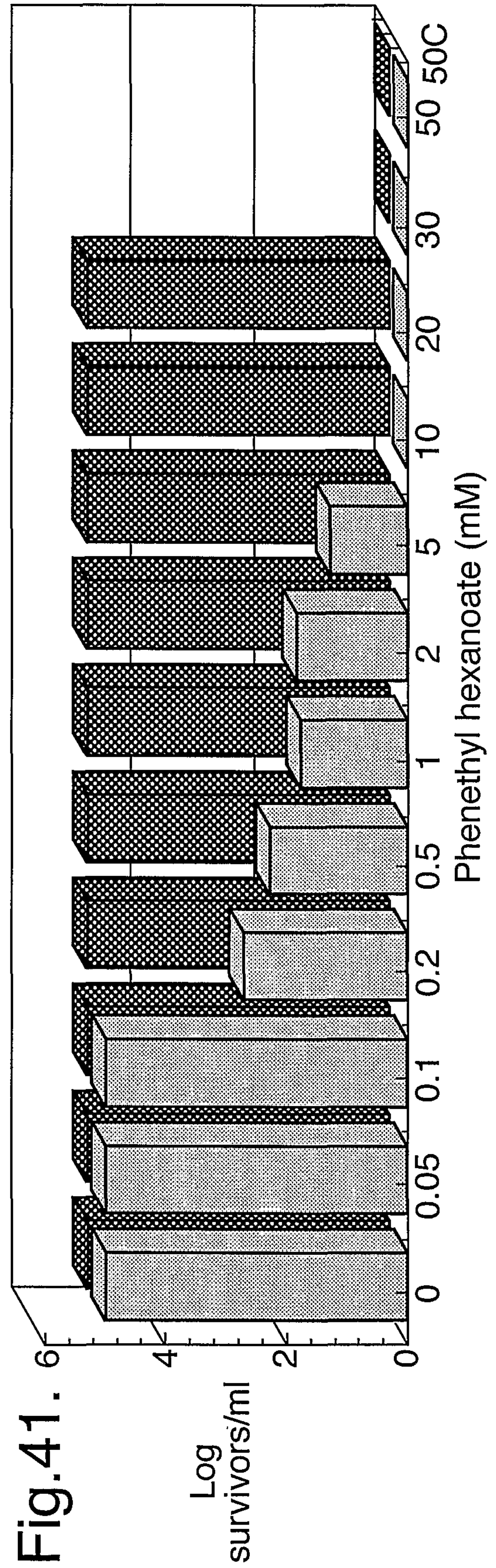
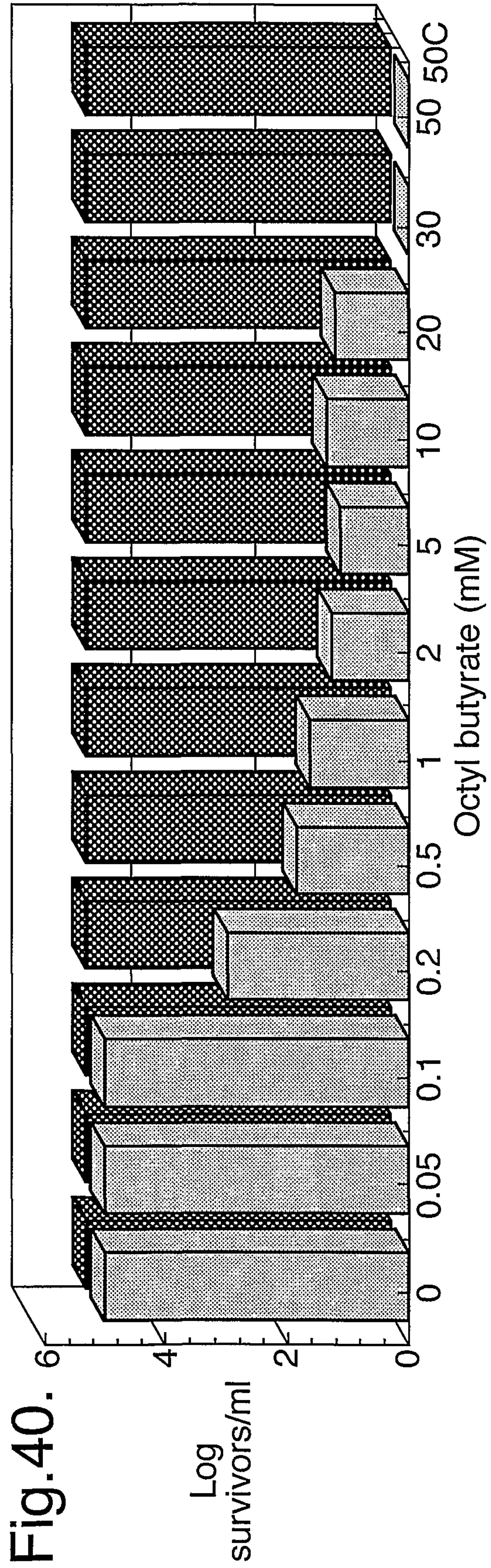
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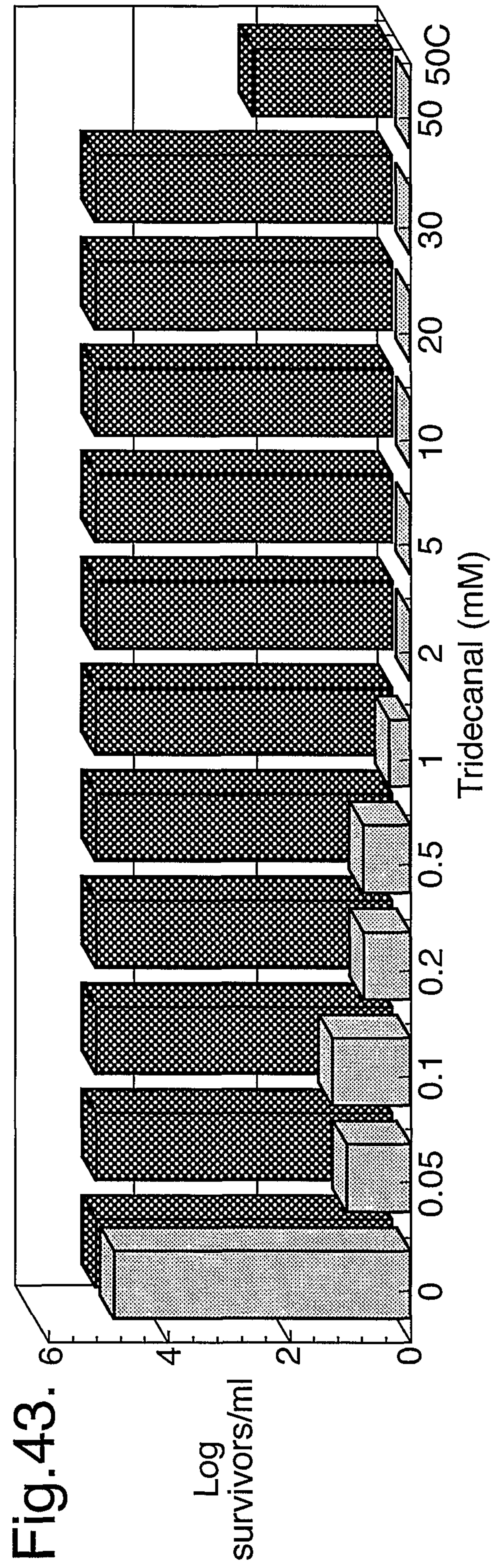
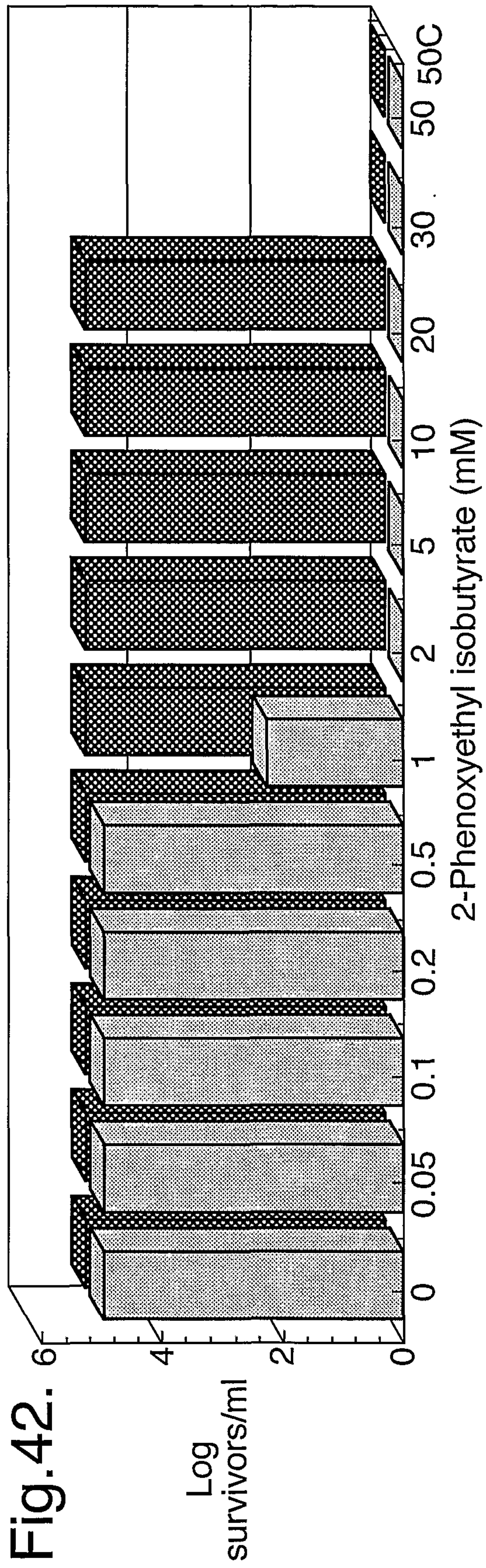
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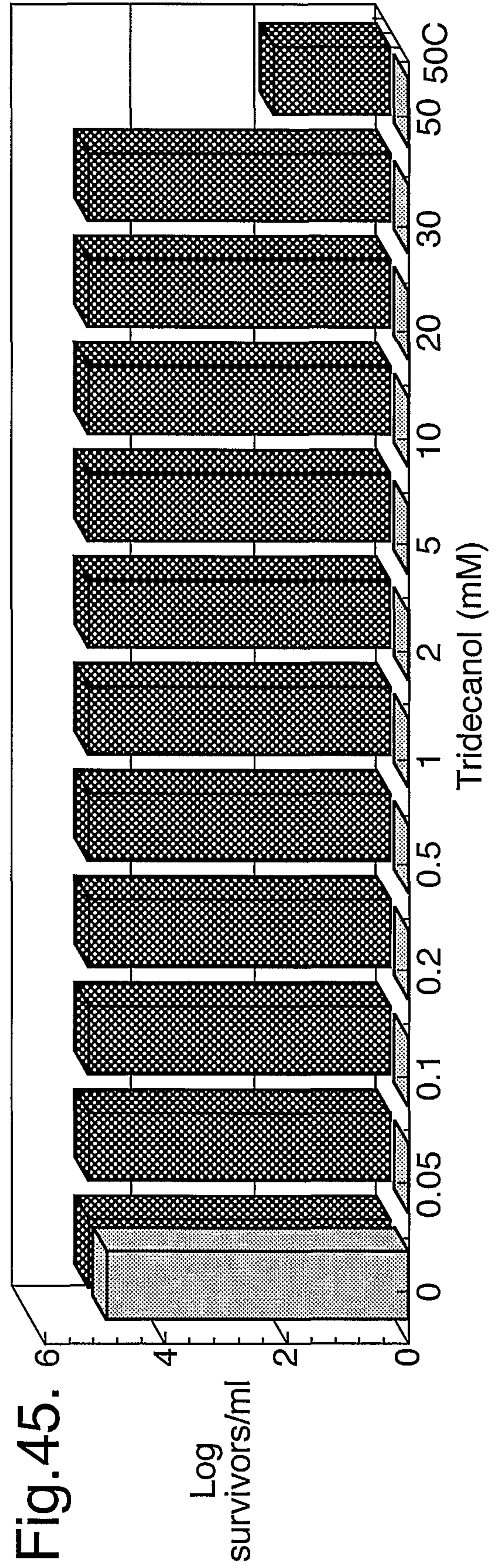
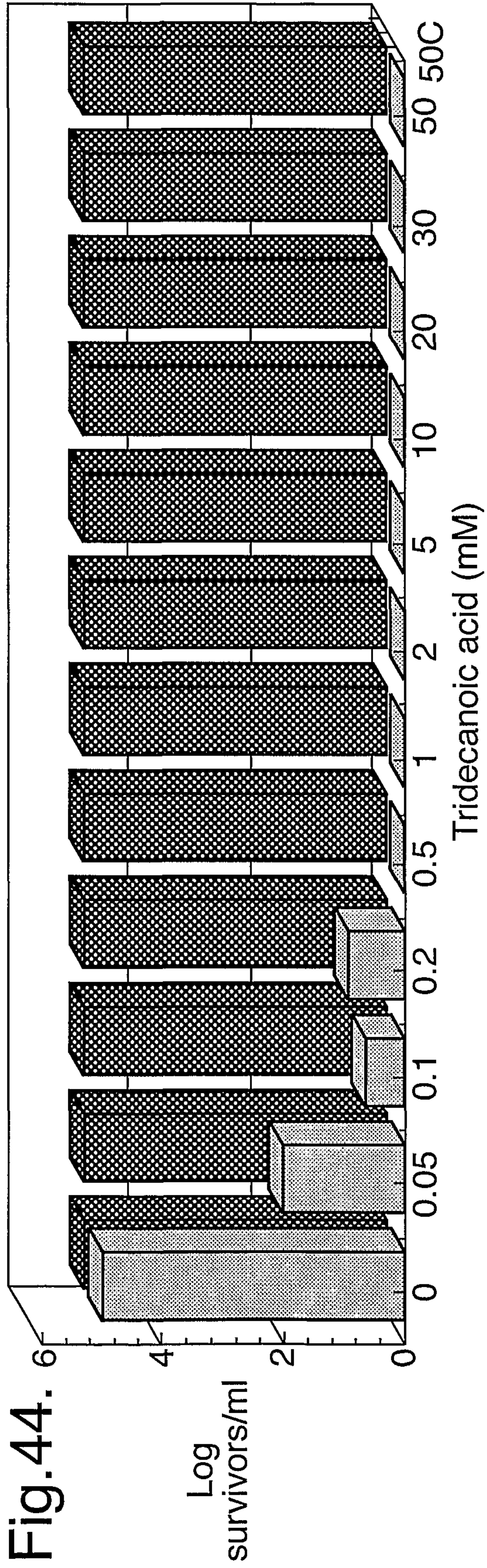
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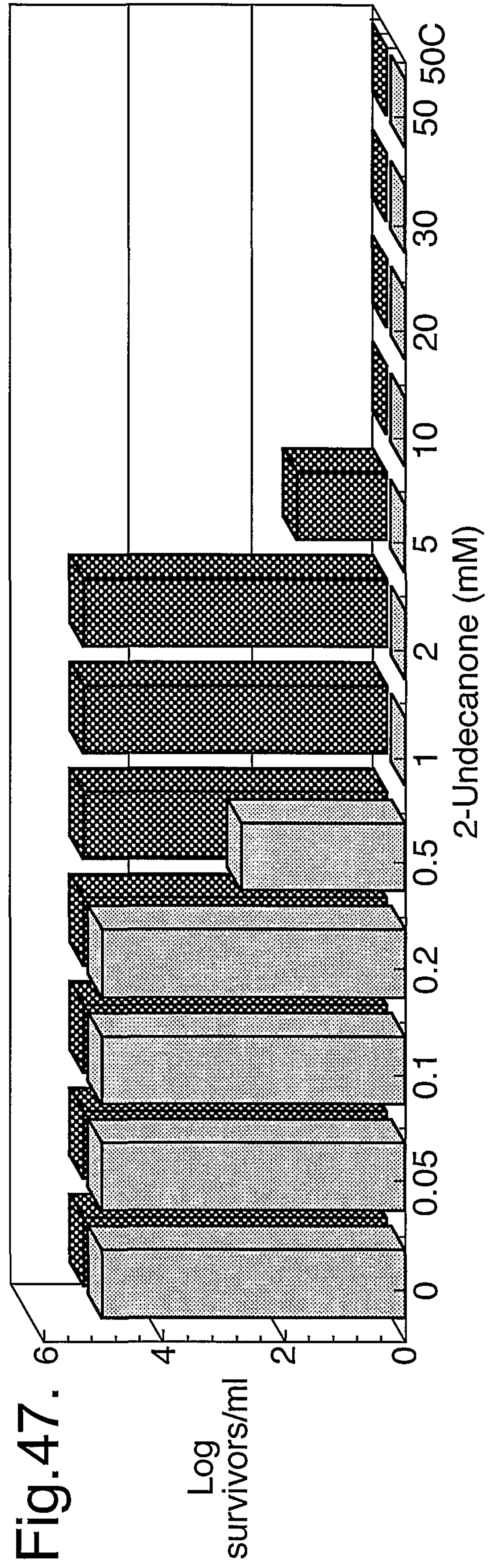
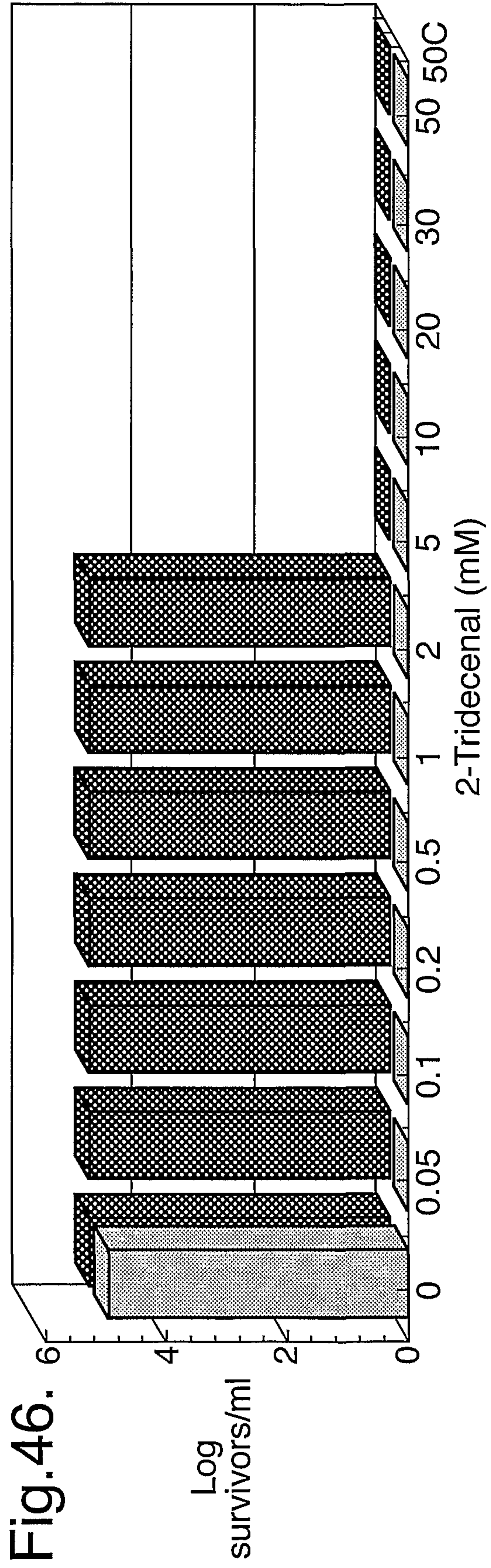
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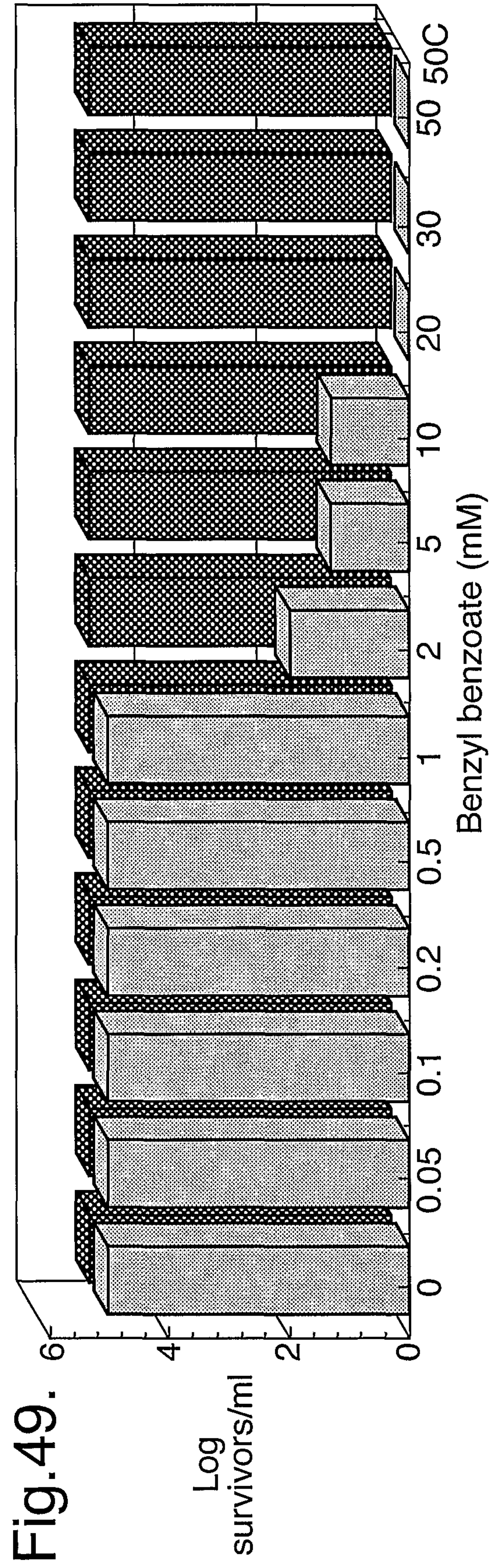
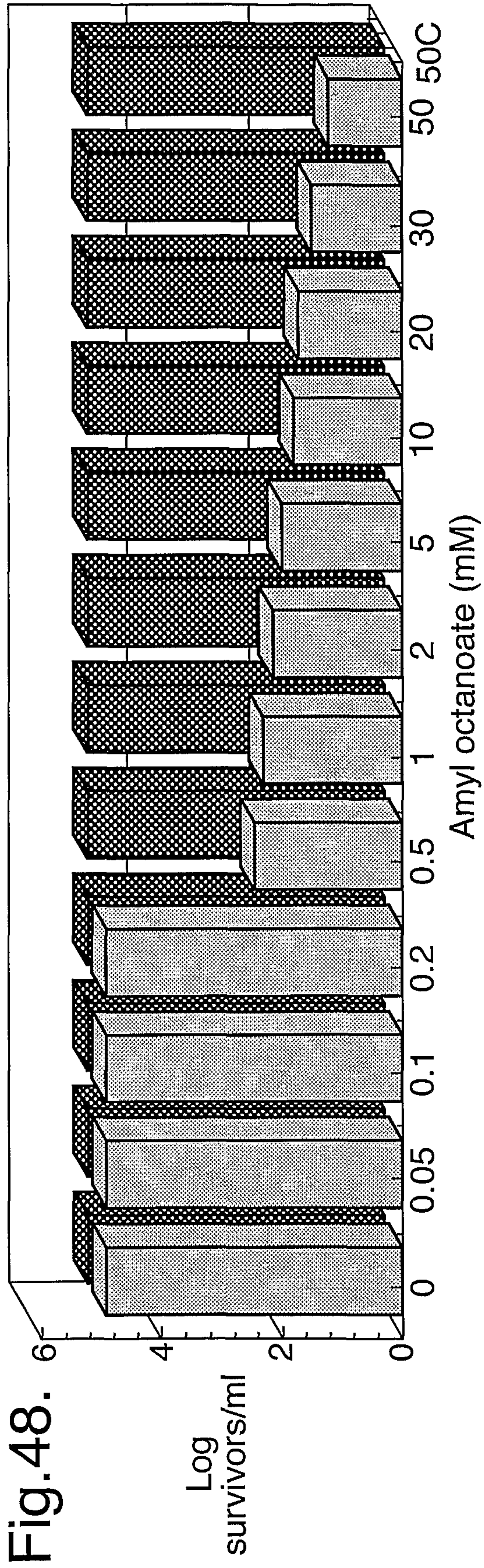
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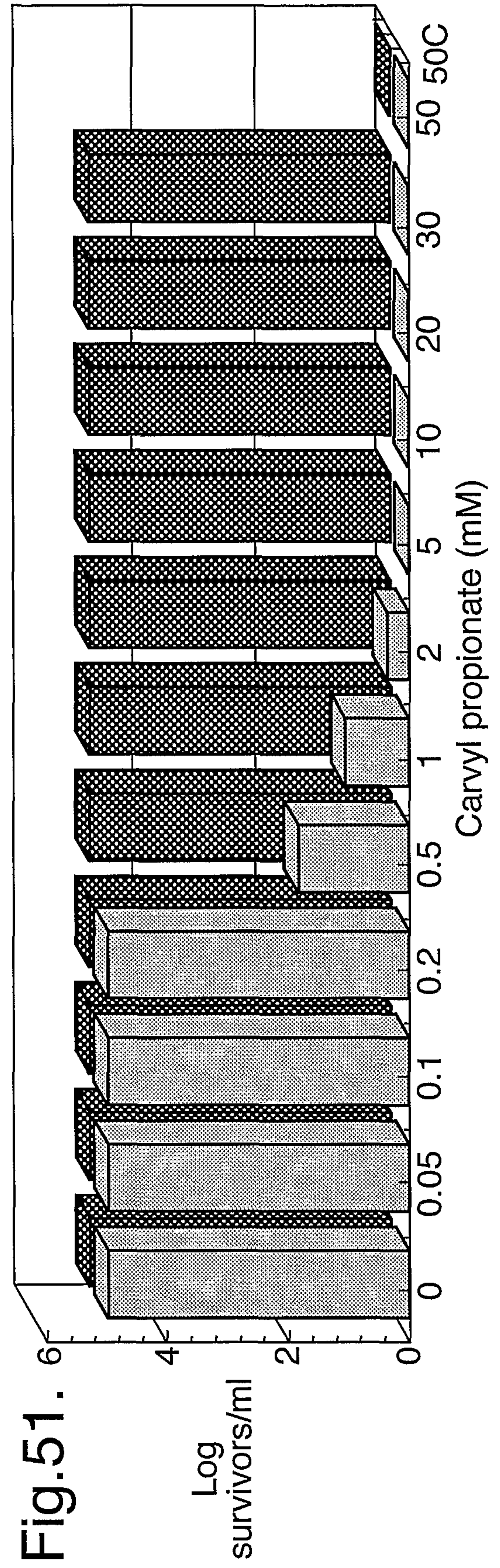
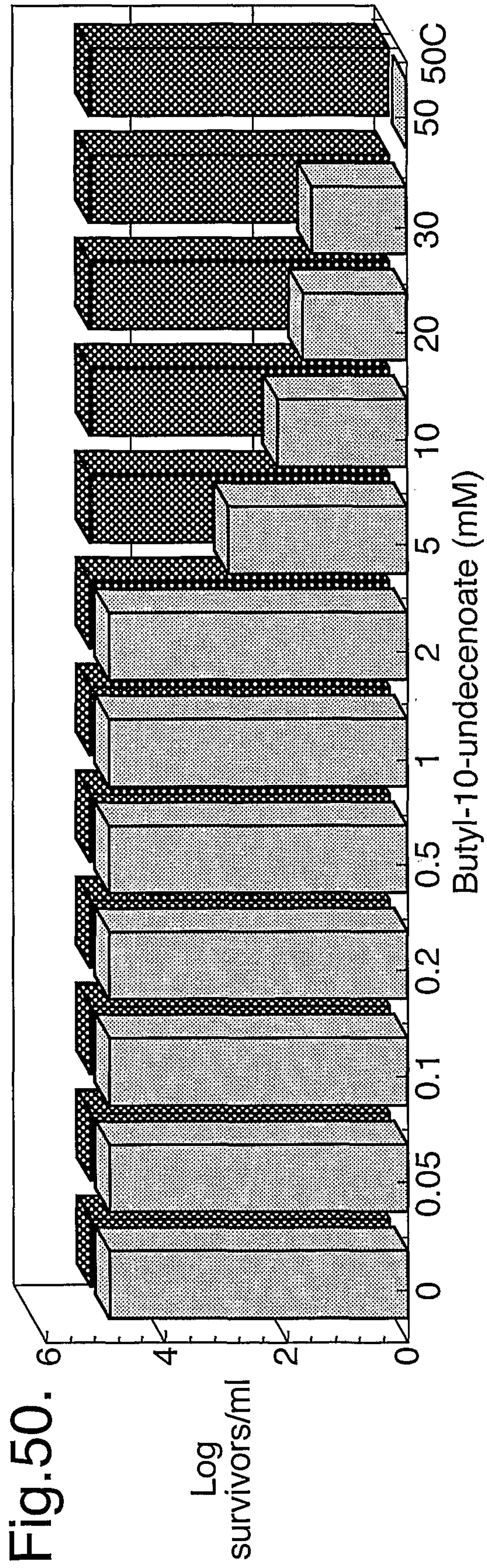
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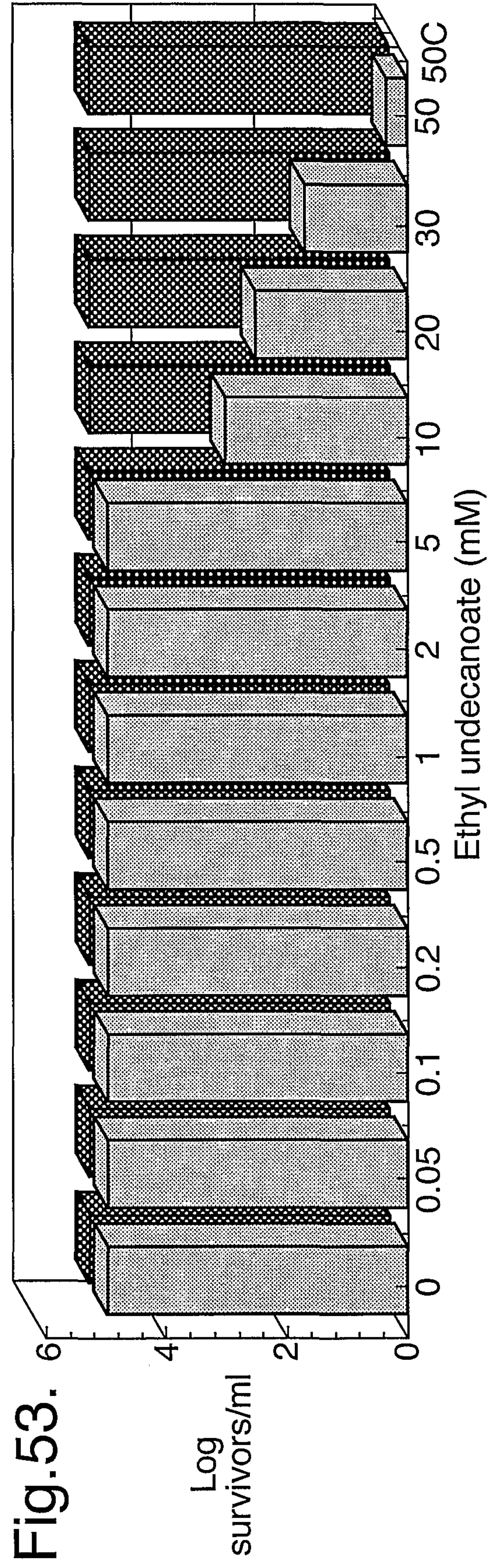
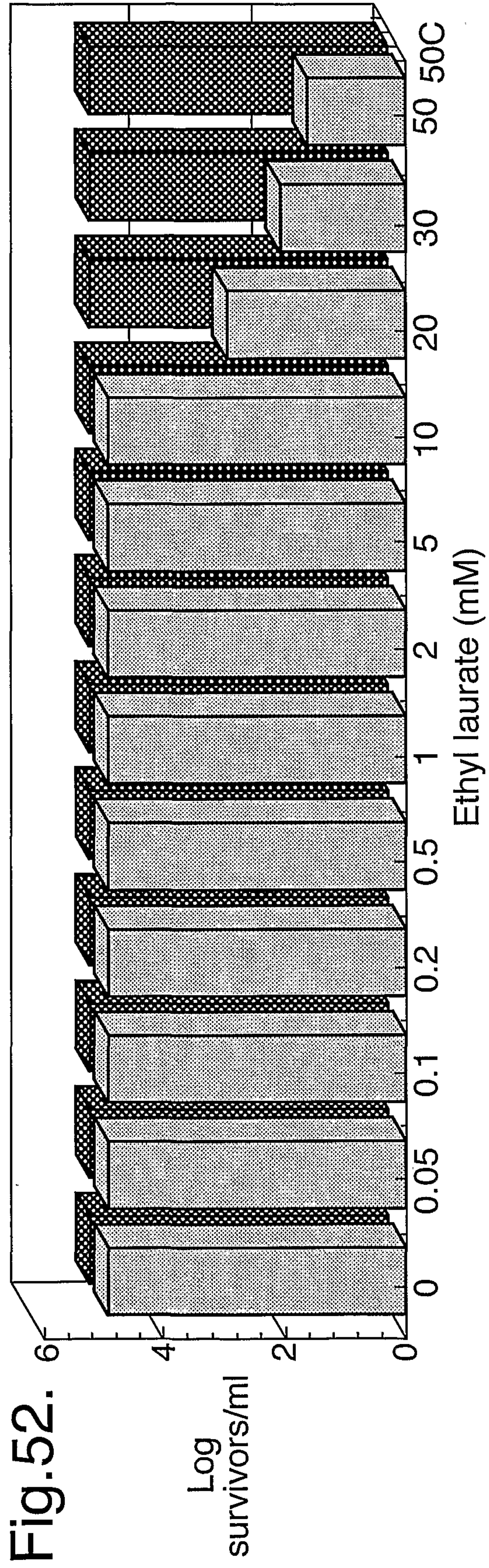
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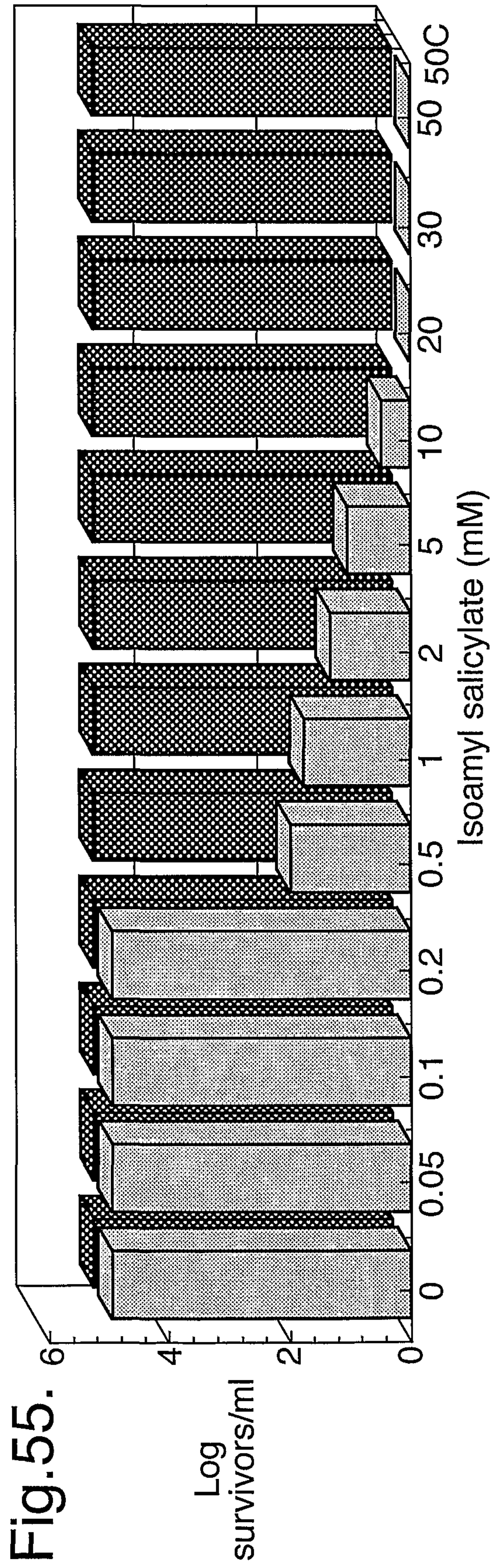
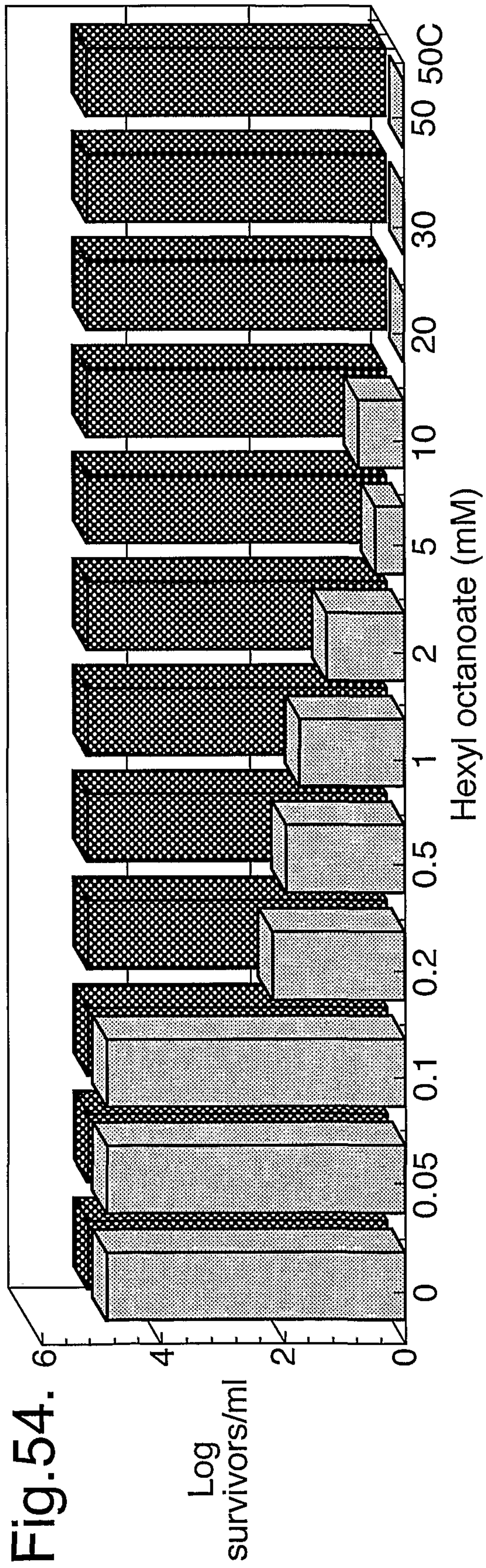
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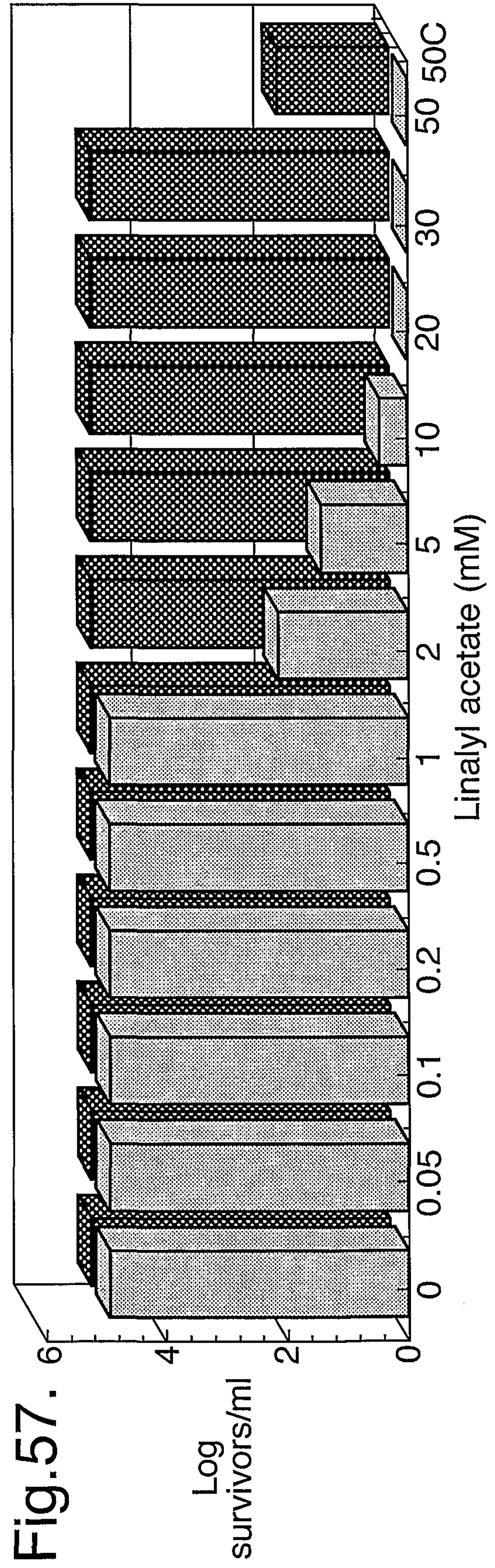
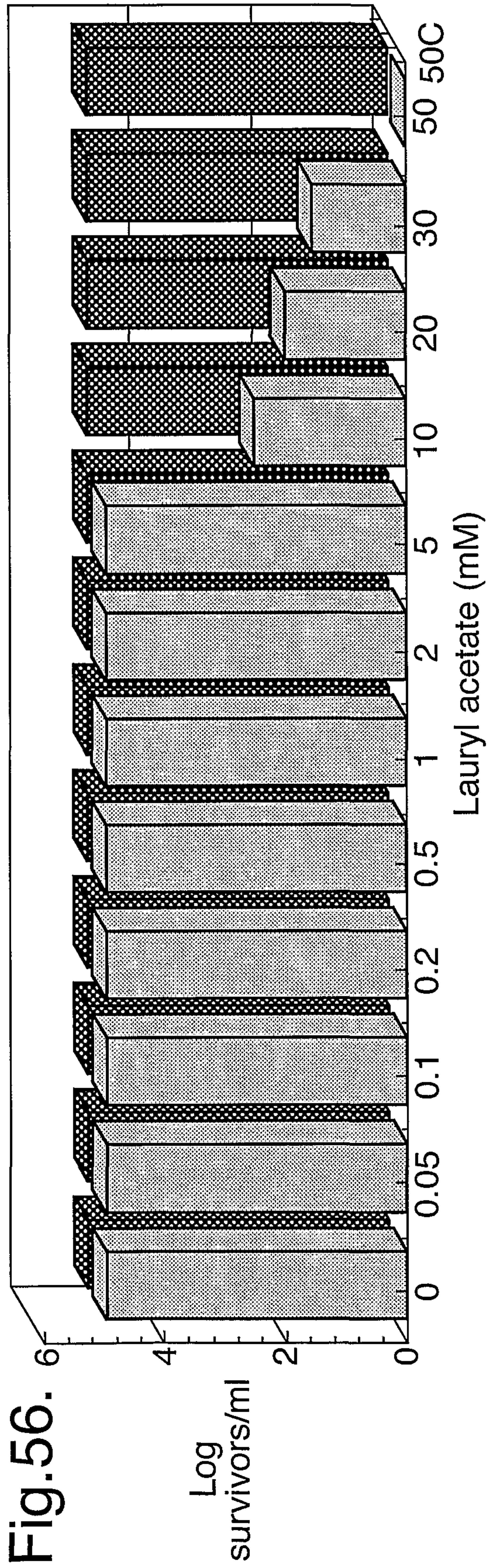
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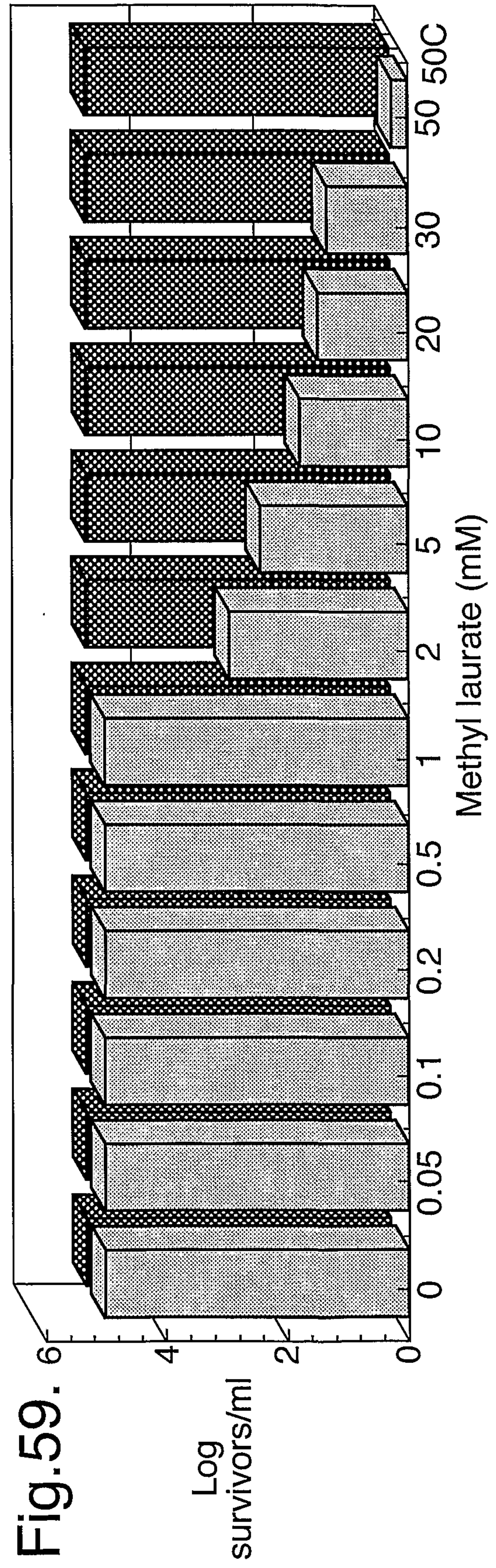
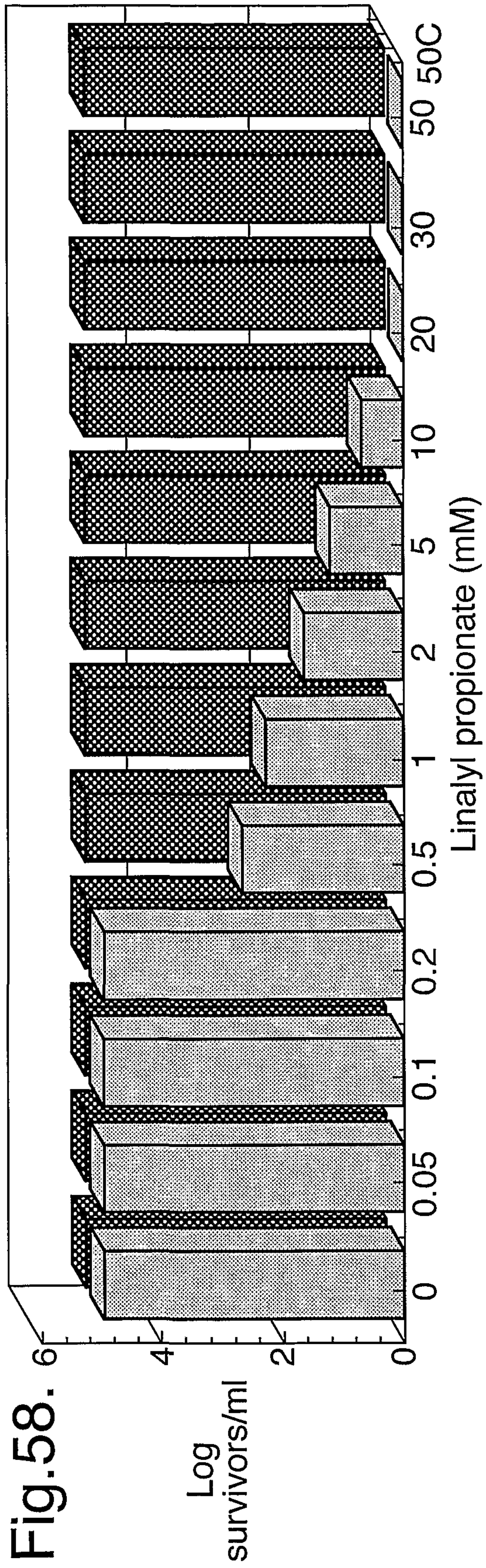
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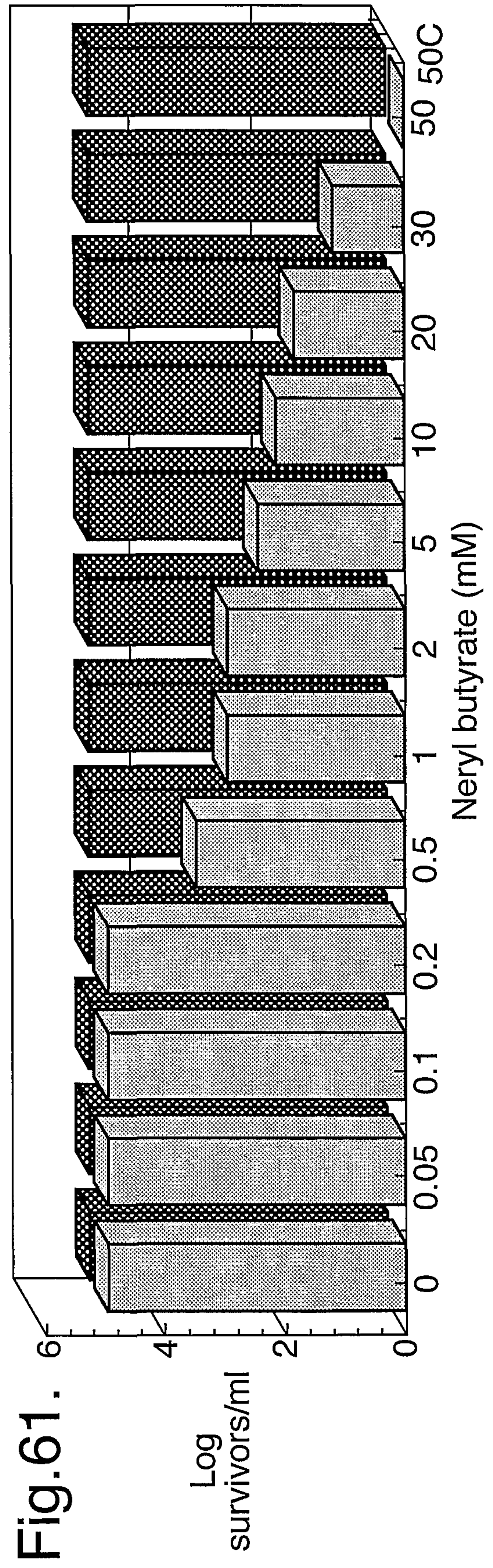
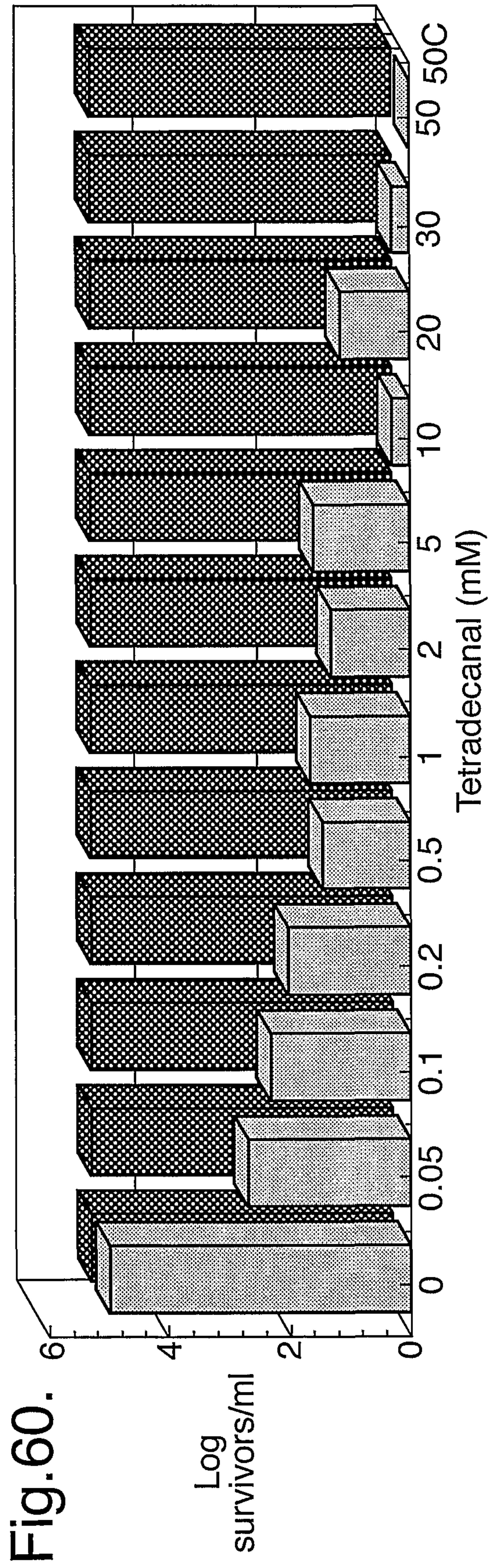
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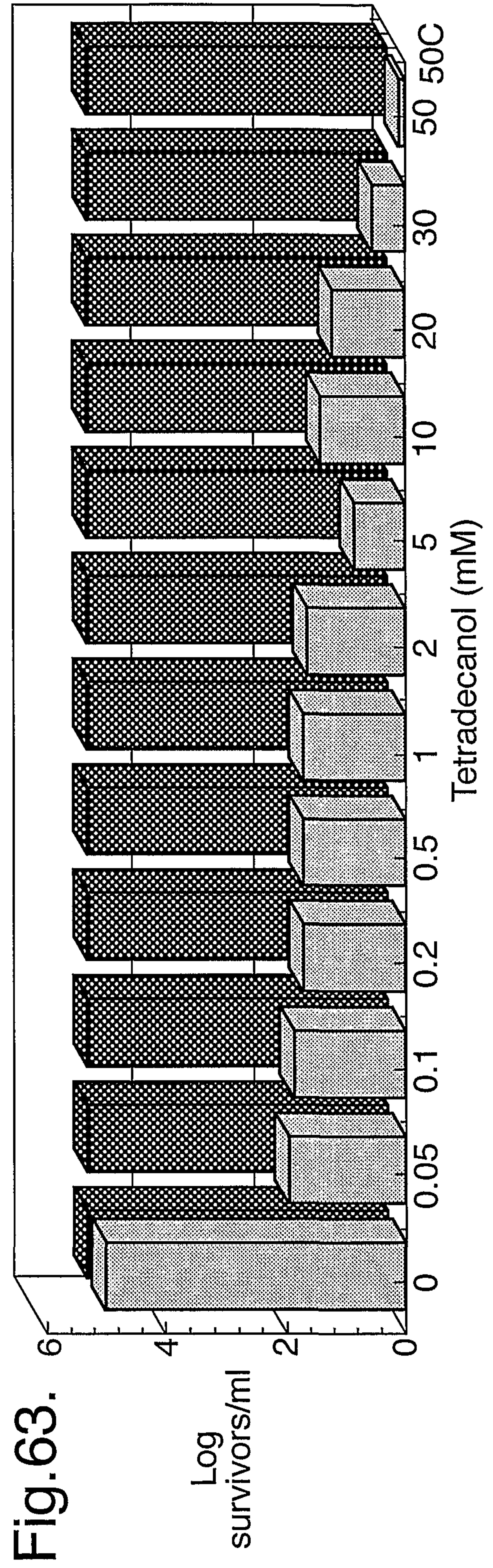
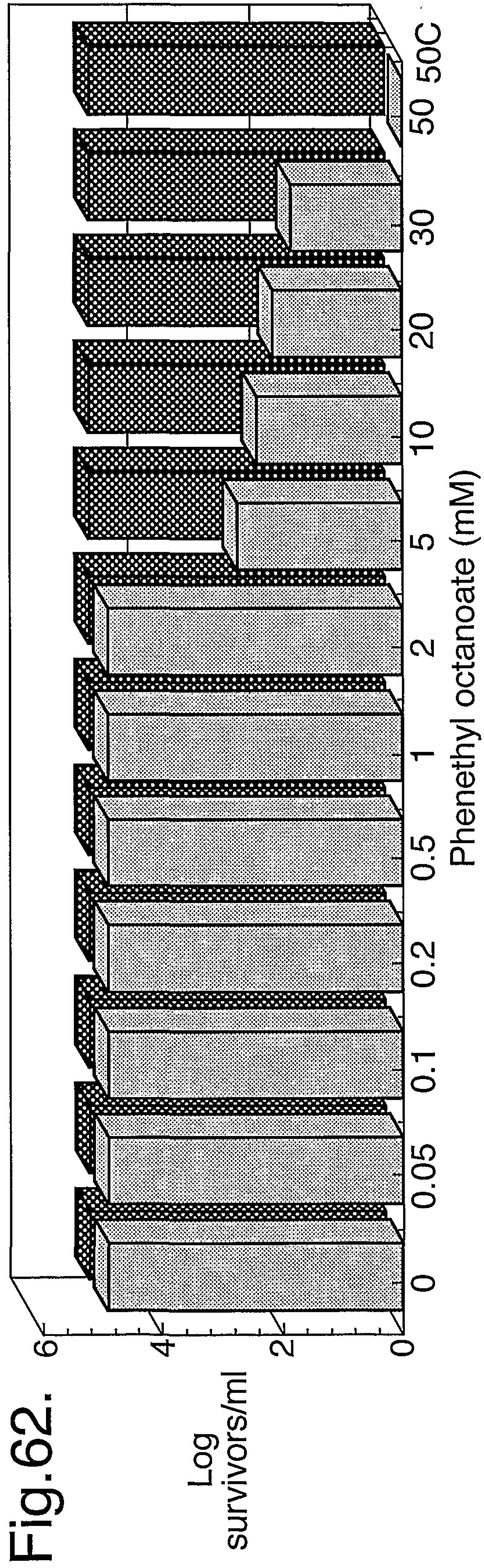
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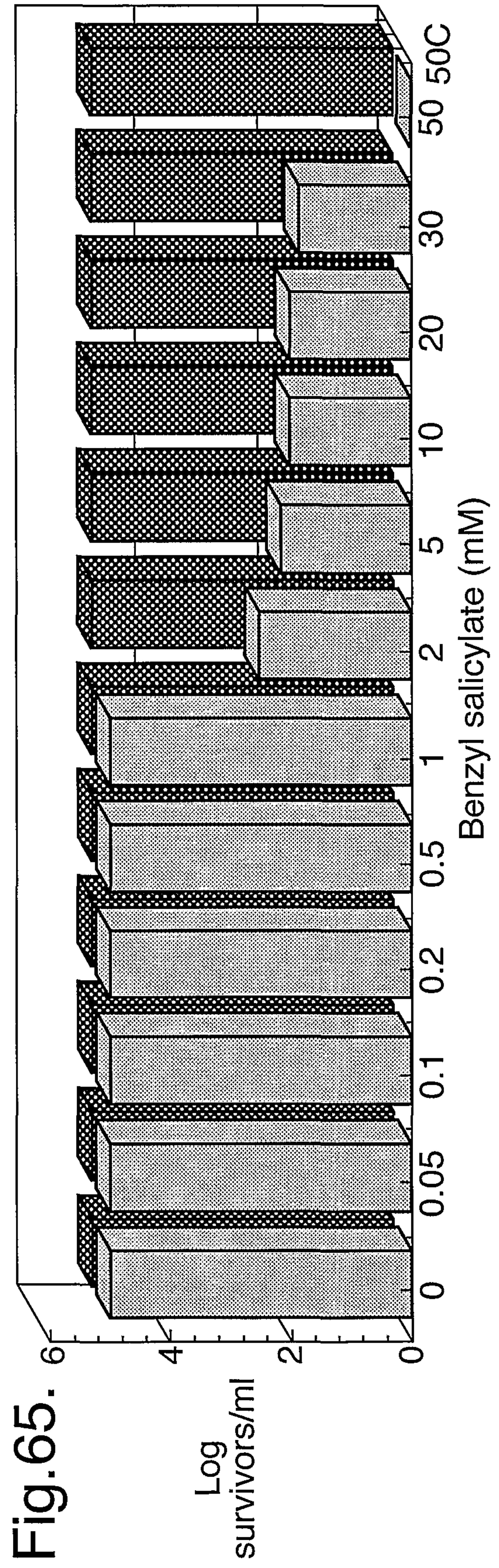
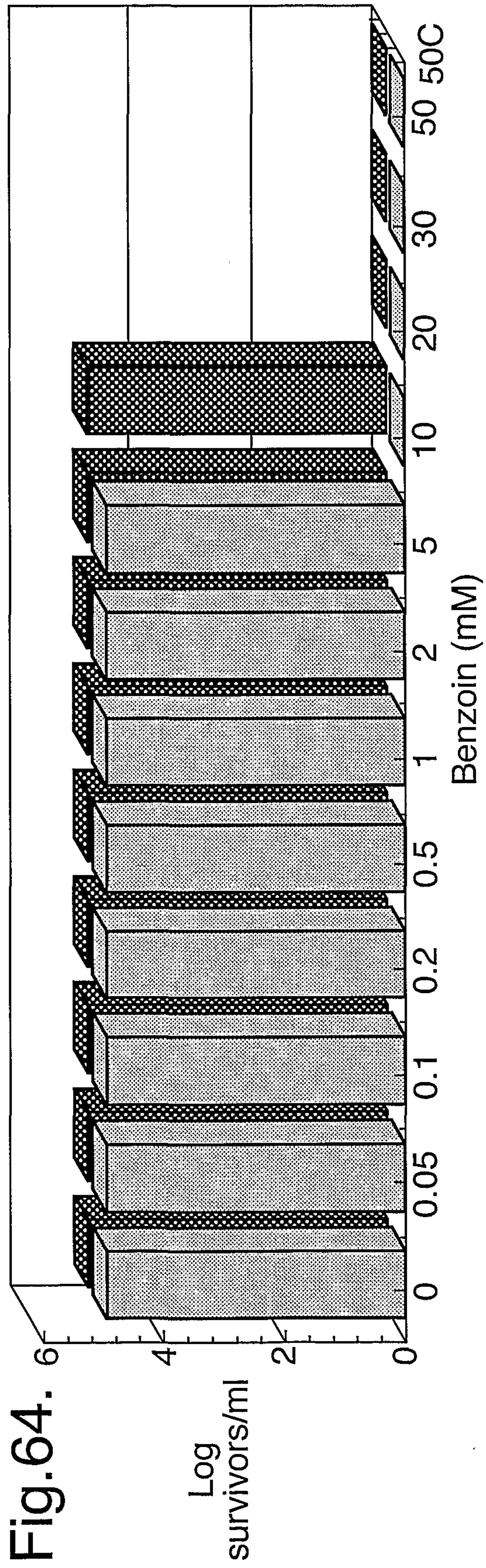
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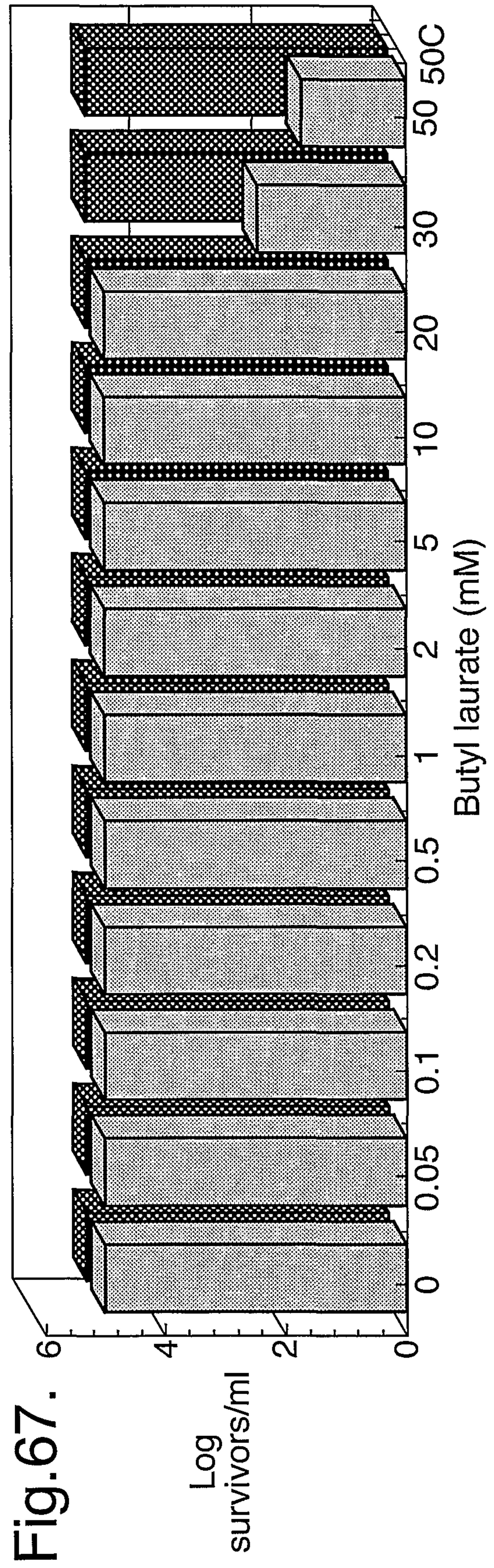
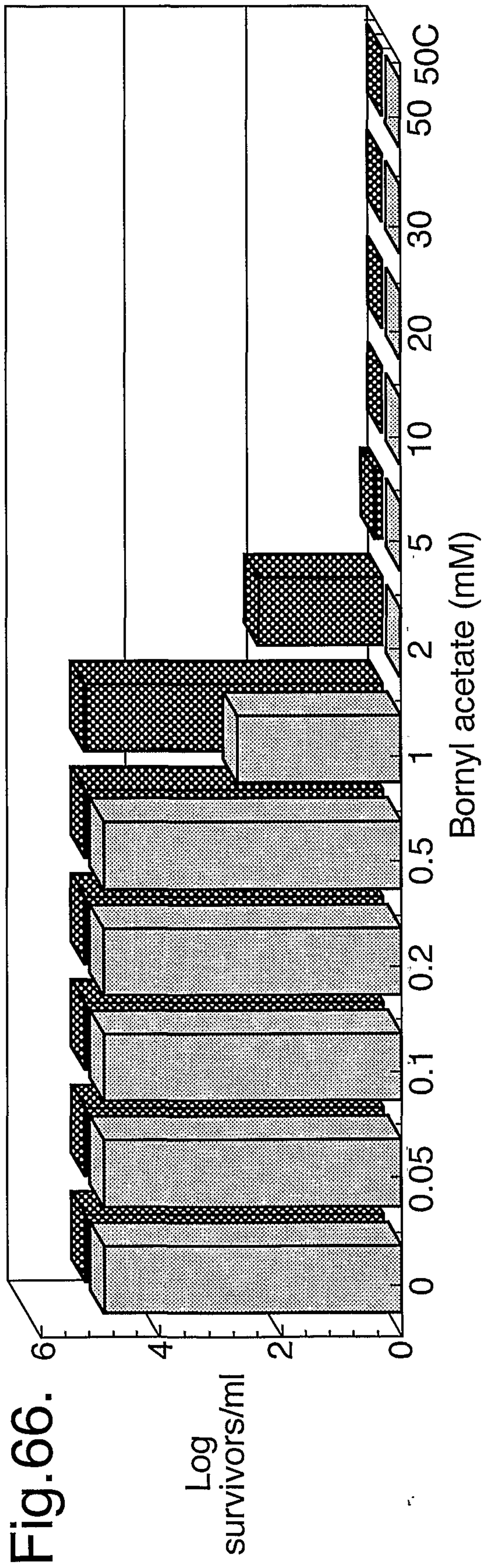
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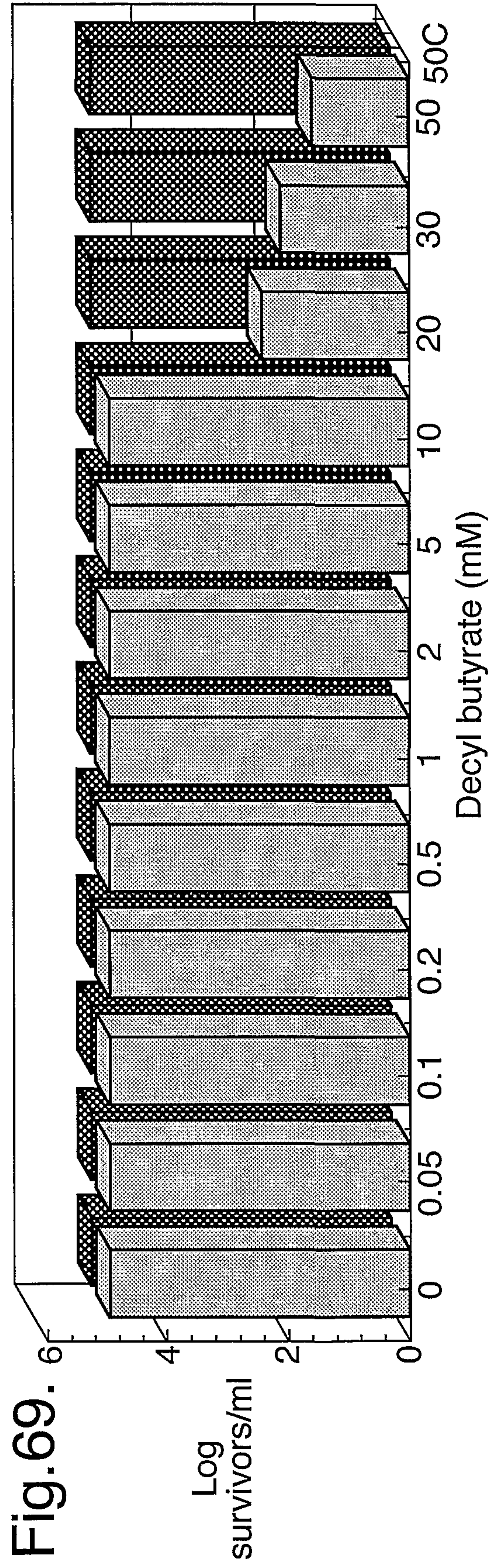
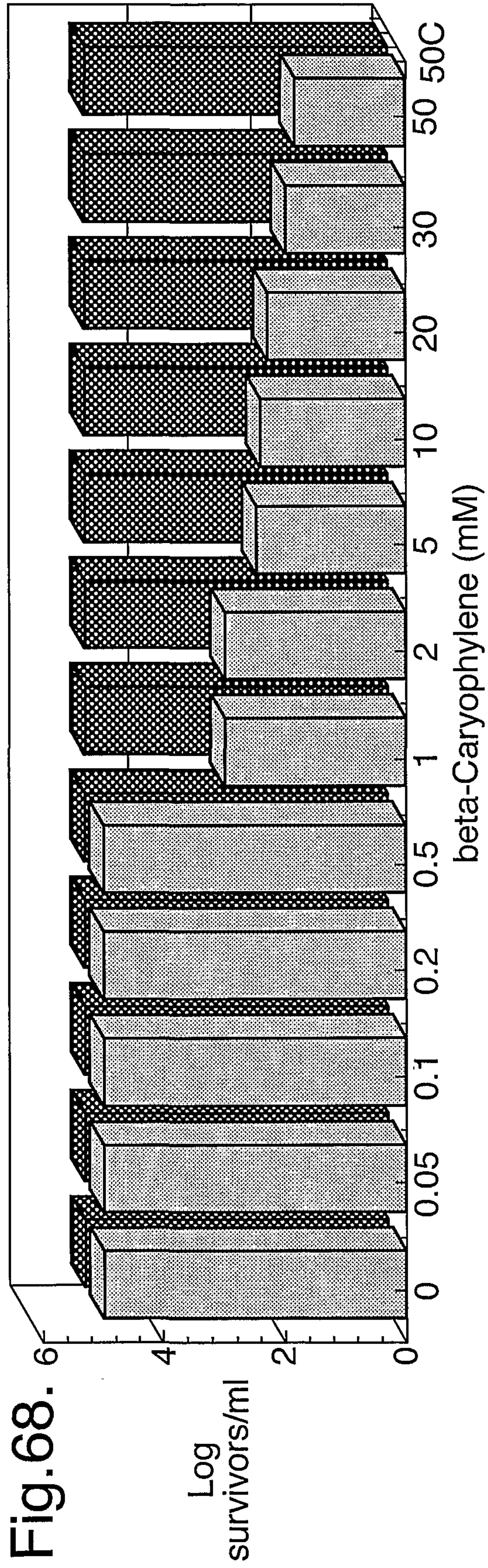
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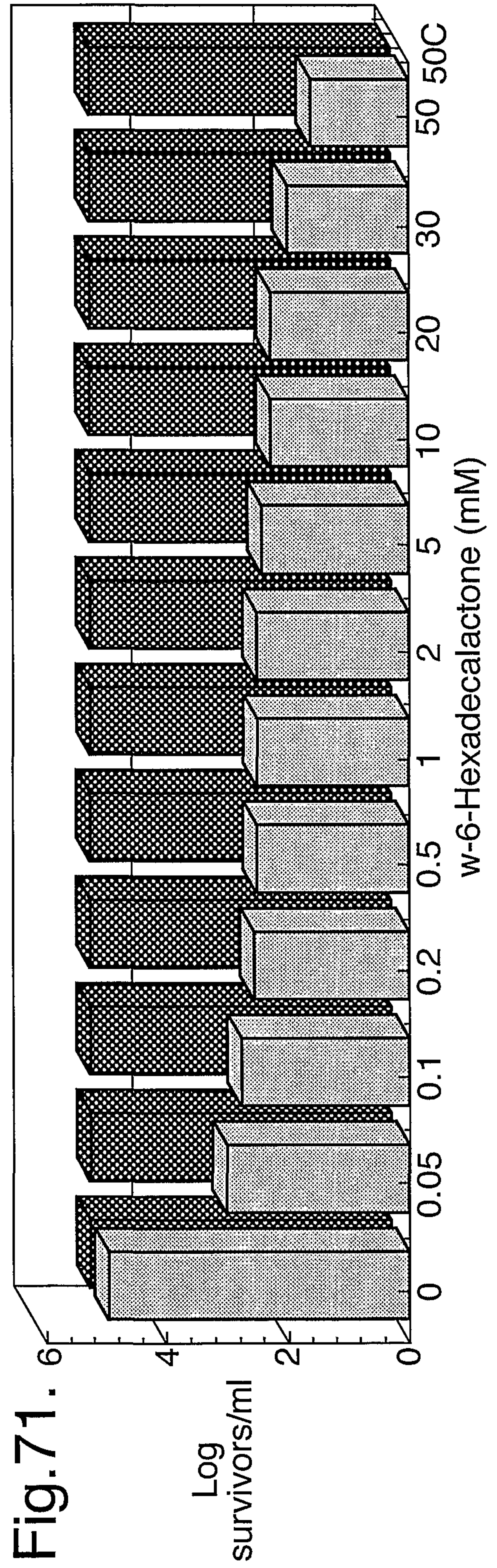
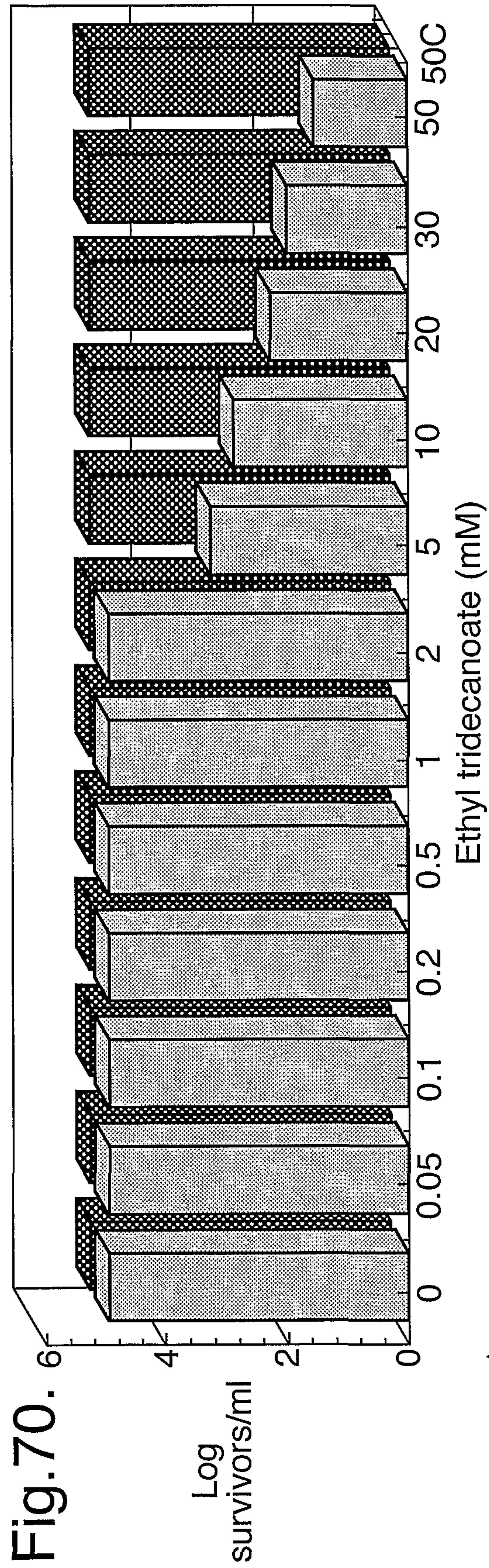
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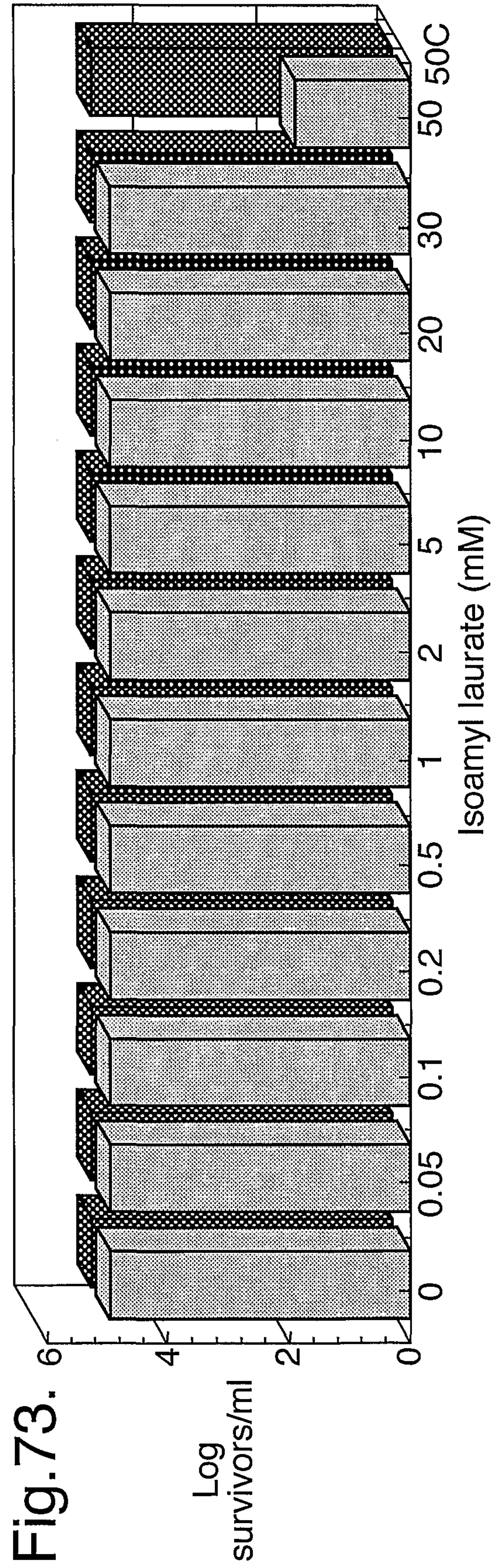
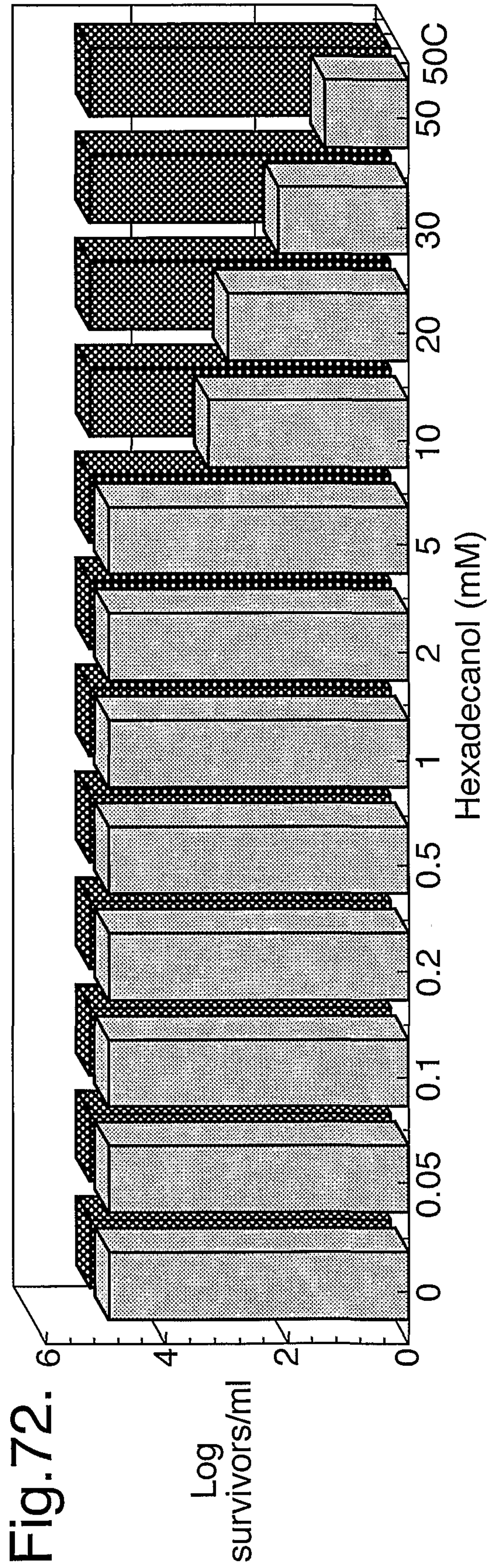
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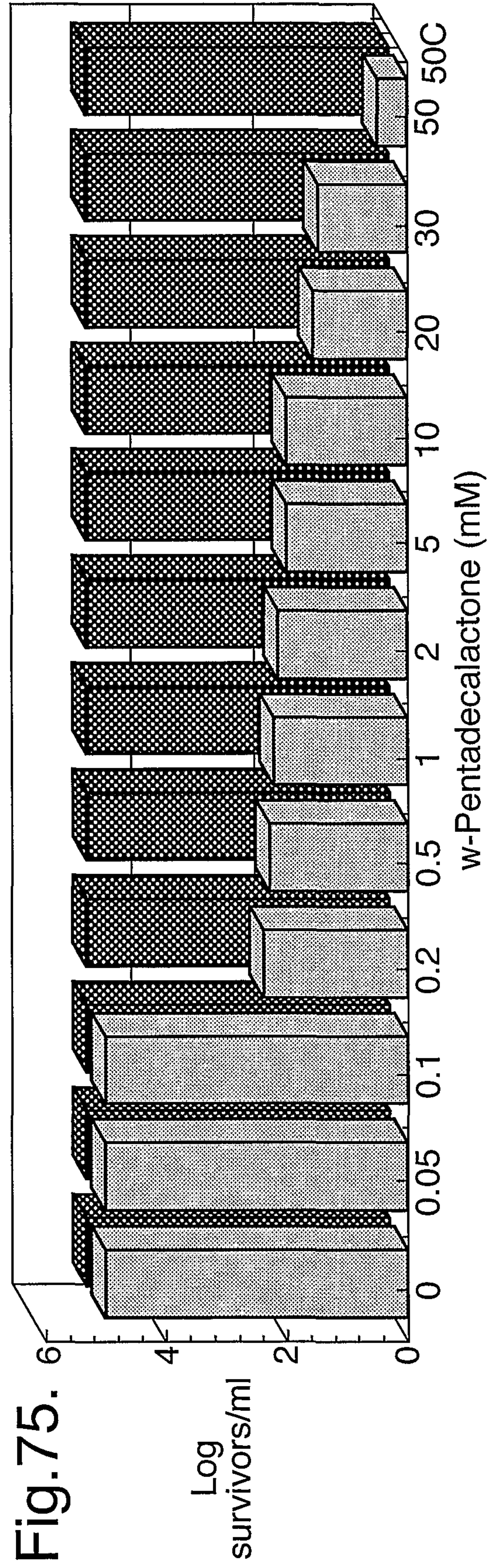
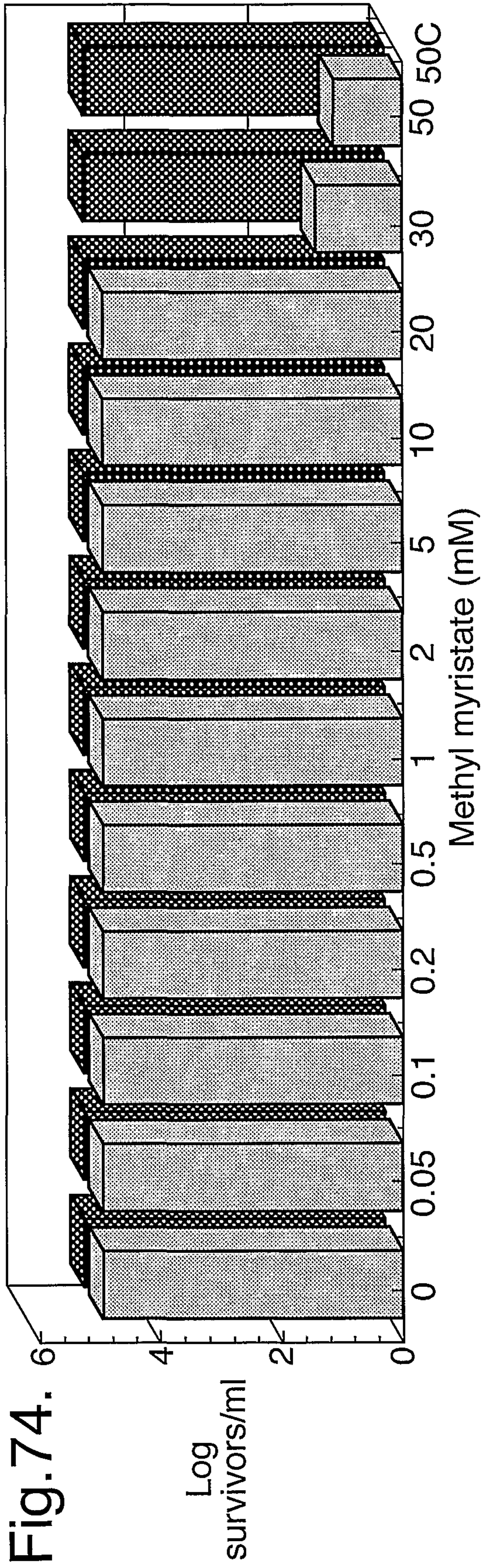
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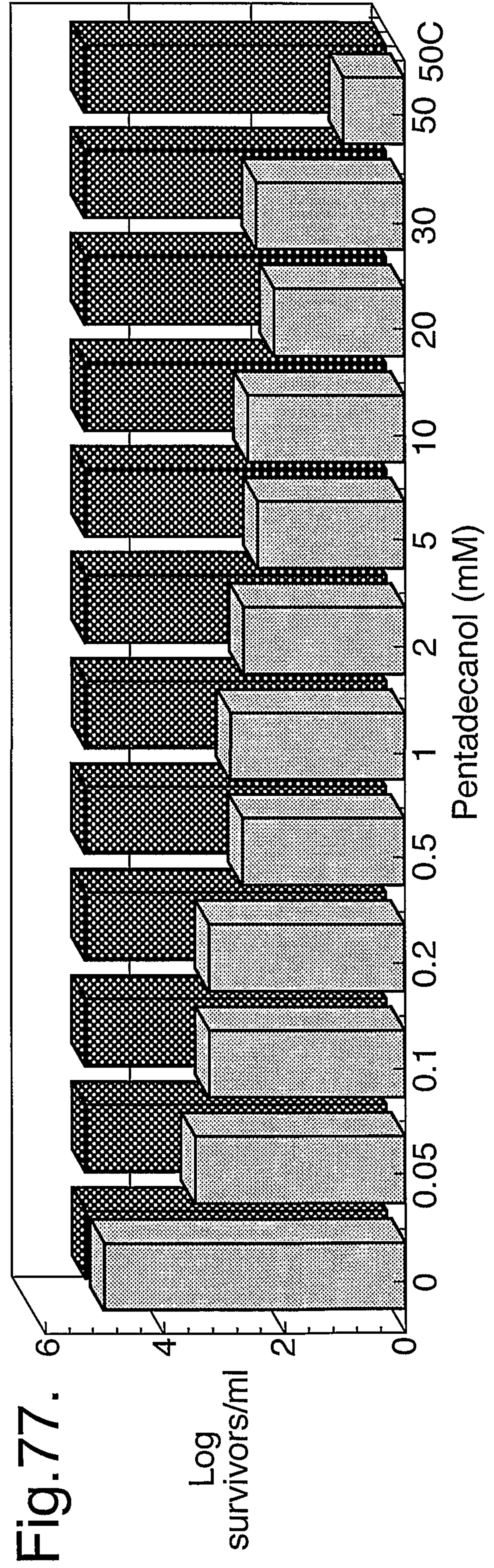
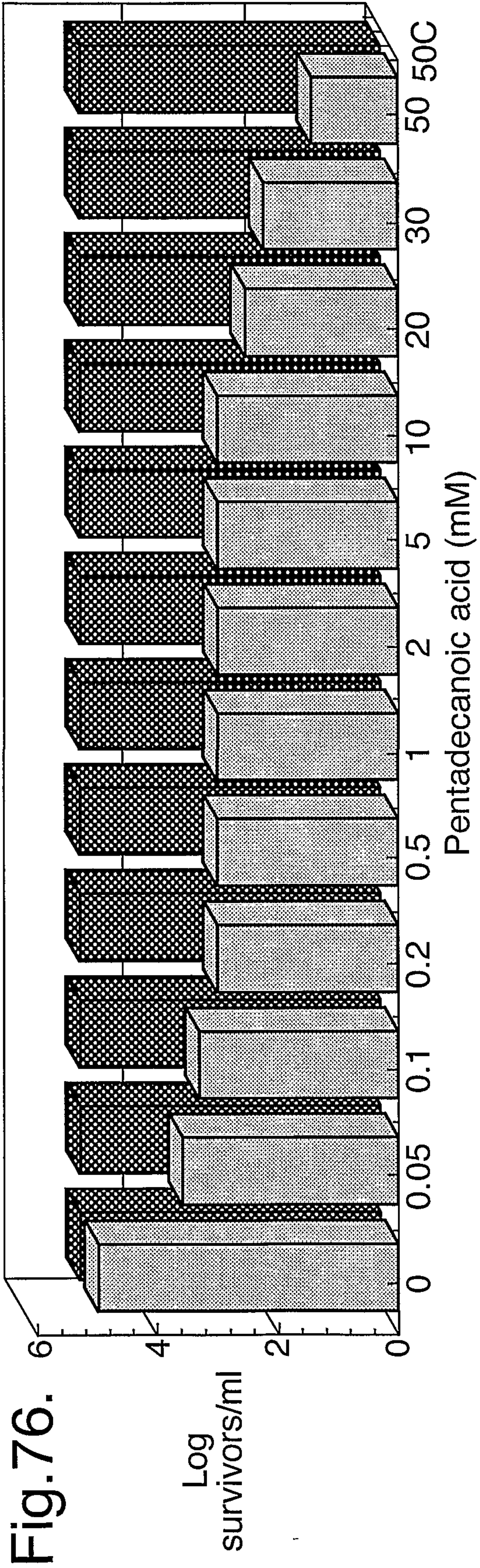
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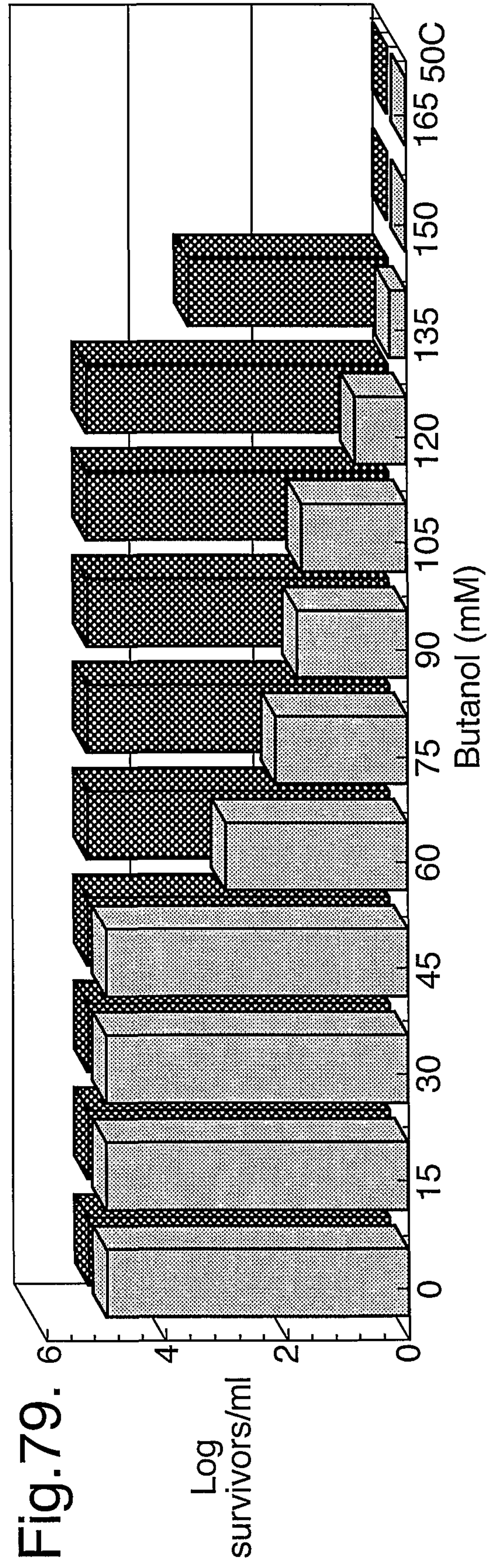
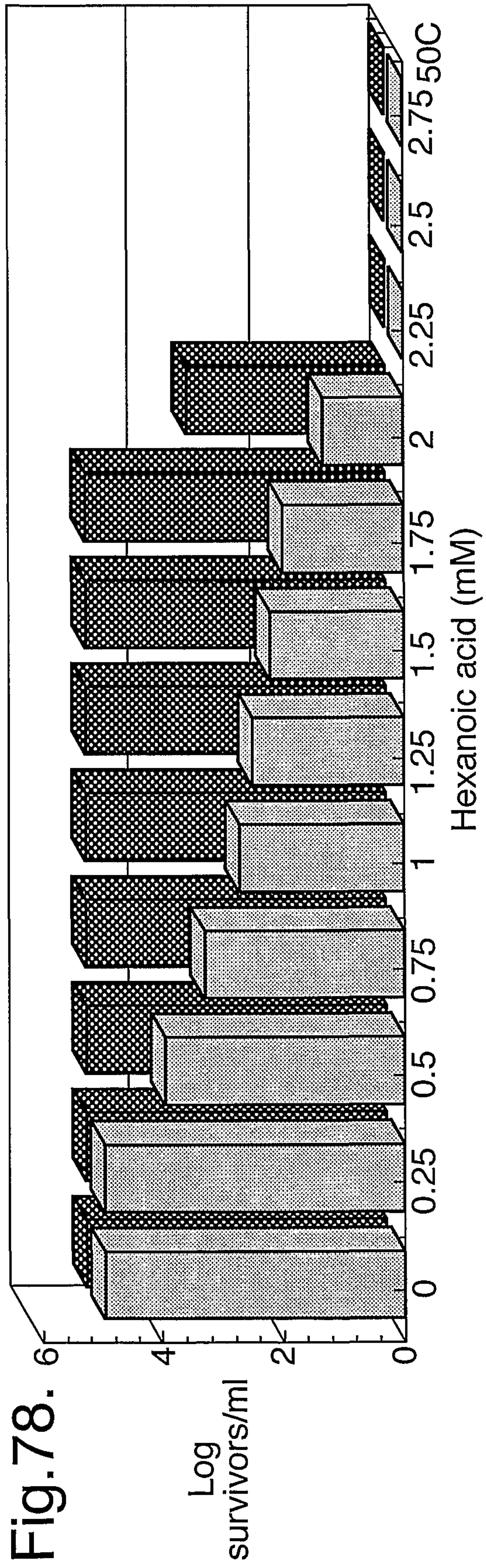
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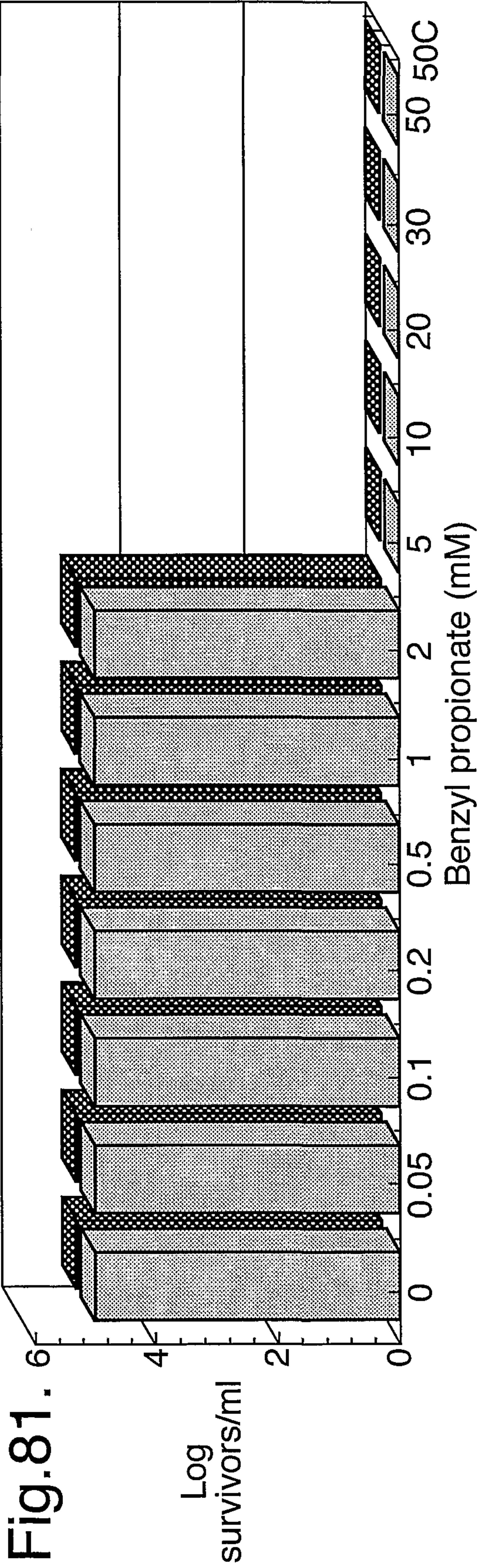
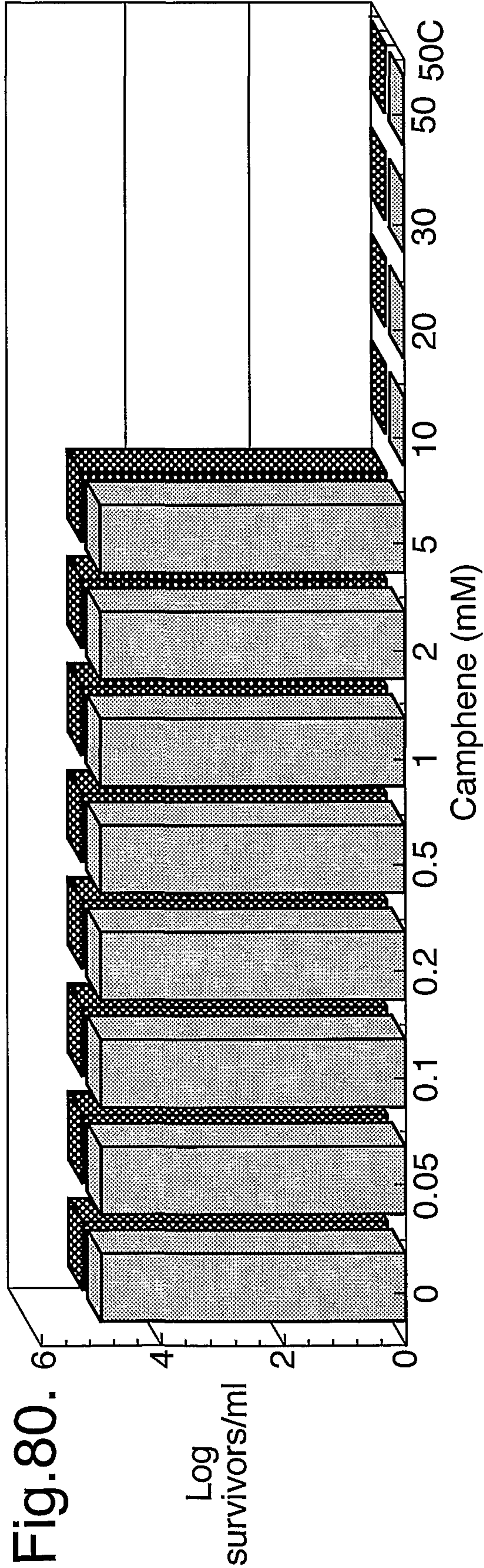


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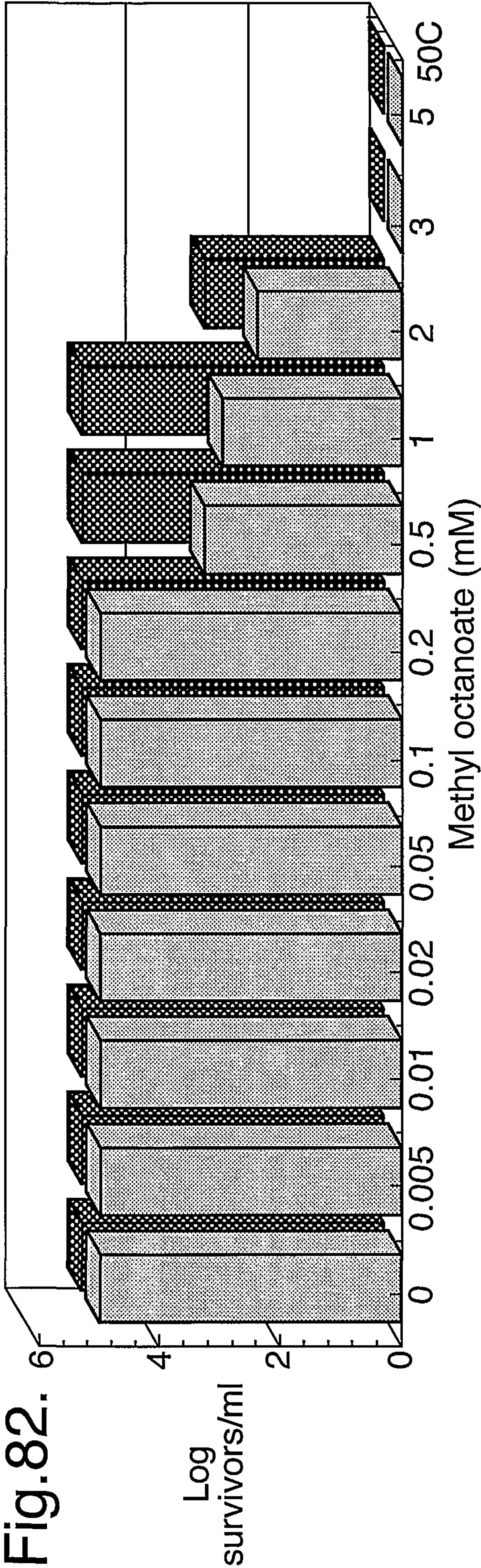


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