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(54) **METHODE D'OXYDATION MICROBIOLOGIQUE DES GROUPEMENTS METHYLE DES COMPOSES HETEROCYCLIQUES**

(54) **MICROBIOLOGICAL OXIDATION OF METHYL GROUPS IN HETEROCYCLES**

(57) A microbiological process is disclosed for the oxidation of methyl groups in aromatic 5- and 6-member ring heterocycles to form the corresponding carboxylic acids. The heterocycle serves as the substrate and is unsubstituted on the carbon atoms adjacent to the methyl group to be oxidized. The reaction of the heterocycle takes place by means of microorganisms of the genus Pseudomonas utilizing toluene, xylene, or cymene. The enzymes of the microorganisms have been previously induced.

ABSTRACT OF THE DISCLOSURE

A microbiological process is disclosed for the oxidation of methyl groups in aromatic 5- and 6-member ring heterocycles to form the corresponding carboxylic acids. The heterocycle serves as the substrate and is unsubstituted on the carbon atoms adjacent to the methyl group to be oxidized. The reaction of the heterocycle takes place by means of microorganisms of the genus Pseudomonas utilizing toluene, xylene, or cymene. The enzymes of the microorganisms have been previously induced.

This invention relates to a new microbiological process for oxidation of methyl groups in aromatic 5- and 6-member ring heterocycles to form the corresponding carboxylic acids. The heterocycle (is unsubstituted) on the carbon atom adjacent to the methyl group to be oxidized. The heterocycle serves as substrate for the reaction which is performed by microorganisms of the genus Pseudomonas utilizing toluene, xylene or cymene, whose enzymes have been previously induced.

These heterocyclic carboxylic acids are important intermediate products for the product of pharmaceutical agents. For example, nicotinic acid (3-pyridine carboxylic acid) is an important intermediate product for the production of nicotinic acid amide, which is a vitamin of the B group and has an essential importance for the nutrition of man and animals [Ullmann, Vol. 19, (1980), p. 603]. 2-Pyrazine carboxylic acid, e.g., is an important intermediate product for the production of the tuberculostatic agent pyrazinamide (2-pyrazine carboxylic acid amide), [Roemps Chemie Lexikon, Vol. 5, (1987)]. 4-Thiazole carboxylic acid serves for the production of thiabendazole, a highly effective antihelminthic (helminthicide), which in turn is a starting material for other newer antihelminthic agents, such as cambendazole [Ullmann, Vol. 23, (1980), p. 46]. 2-Thiophene carboxylic acid exhibits an antiallergic effect [Ullmann, Vol. 23, (1980), p. 219].

In-depth investigation of the oxidation of methyl groups has so far been conducted with aromatic hydrocarbons.

The production of carboxylic acids by the microbial oxidation of methylated aromatic compounds was exhaustively described in the works of Raymond et al. [Raymond et al., Process Biochem., (1969), pp. 71 to 74]. U.S. Patent No. 3,393,289 describes a process for the biochemical oxidation of methyl groups in aromatic

hydrocarbons with a gram-positive microorganism strain belonging to the genus Nocardia. Drawbacks of these processes include the fact that, for example, in the methyl group oxidation of aromatic hydrocarbons the benzene ring of the corresponding acid is cleaved off.

In regard to the use of Pseudomonas putida ATCC No. 33015, it is known that the biochemical oxidation of the methyl group of toluene to form benzoic acid takes place in three steps. By the action of toluene monoxygenase benzyl alcohol first results, which is then in two other steps, catalyzed by an alcohol dehydrogenase and aldehyde dehydrogenase, reacted to form the acid. In this strain, both the Xyl gene, which codes for enzymes of xylene degradation, and the genes which are responsible for the regulation of the Xyl gene, lie on the plasmid pWWO. This archetypal Tol plasmid has already been thoroughly studied in a molecular biological manner [Harayama et al., J. Bacteriol. 171, (1989), pp. 5048 to 5055; Burlage et al., Appl. Environ Microbiol. 55, (1989), pp. 1323 to 1328].

Likewise, microbiological processes for the oxidation of methyl groups of an N-heterocycle are also known from the literature. According to Soviet Union Patent No. 417,468, 2-methyl pyridine may be oxidized with a gram-positive microorganism strain of the genus Nocardia to form the corresponding acid.

Soviet Union Patent No. 228,688 describes a microbiological process for the production of nicotinic acid from 3-methyl pyridine using a gram-positive microorganism of the genus Mycobacterium. A microbiological process for the production of nicotinic acid with gram-positive bacteria of the genus Nocardia is known from Soviet Union Patent No. 302,341.

The drawbacks of methyl group oxidation of N-heterocycles with gram-positive bacteria include the fact that, with such alkane-utilizing bacteria, the mixture

ratio of the alkane to the substance to be oxidized has to be adjusted exactly to achieve a biotransformation and that no biotransformation of the substrate occurs in the absence of the alkane, i.e. the alkane used for the induction must always be present, also in the reaction of the substrate. By comparative tests with the gram-positive bacterium Nocardia and applicants' gram-negative Pseudomonas, it was clearly shown that using Nocardia even in the presence of an alkane, such as dodecane, 3-methyl pyridine could not be oxidized to nicotinic acid.

In addition, U.S. Patent No. 4,859,592 describes a process for the production of picolinic acid using Pseudomonas putida by an alkyl-substituted aromatic hydrocarbon being formed in the presence of molecular oxygen in a first step by a dioxygenase into a 2-hydroxy muconic acid semialdehyde, and then the latter being reacted in a second step with ammonia or a primary amine to form 2-picolinic acid. The drawback of such process is that the corresponding picolinic acid is formed only in the second step by the reaction of the 2-hydroxy muconic acid semialdehyde with ammonia .

An object of the present invention is to eliminate the above-described prior art drawbacks and to develop a simple, one step process for microbiological methyl group oxidation, from which the corresponding acids can be isolated in good yield and purity and the aromatic heterocycle is not cleaved off. A further object is to provide a process in which the compound used for the induction, after completion of the induction of the enzyme, need no longer be present during the reaction of the substrate and, thus, the reaction does not depend on the amount of the enzyme inducer.

Accordingly, the invention provides a microbiological process for oxidation of methyl groups in aromatic 5- or 6-member ring heterocycles. The

heterocycle should be unsubstituted on the carbon atom adjacent to the methyl group to be oxidized to the corresponding carboxylic acid. The methylated heterocycle is used as substrate for the reaction. The reaction is performed by microorganisms of the genus Pseudomonas, utilizing toluene, xylene or cymene, whose enzymes have been previously induced.

In the accompanying drawings:

Figure 1 is a schematic diagram of the equipment used in a preferred embodiment of the invention; and

Figure 2 is a graph which shows the results of the biotransformation in Figure 1.

The reaction of the process of the invention is suitably performed by microorganisms of the species Pseudomonas putida utilizing toluene, xylene or cymene.

Preferably the xylene-utilizing microorganism strain Pseudomonas putida ATCC No. 33015 or an effective mutant of the latter or the cymene-utilizing microorganism strain Pseudomonas putida DSM 5709 or an effective mutant of the latter is used. The reaction is performed especially with the microorganism strain Pseudomonas putida ATCC No. 33015. The microorganism strain Pseudomonas putida (DSM 5709) has been deposited with the German Collection of Microorganisms (DSM) and Zellkulturen [Cell Cultures] GmbH, Mascheroderweg 1b, 3300 Braunschweig, FRG, on December 22, 1989 under number DSM 5709.

The microorganism strain Pseudomonas Putida ATCC No. 33015 has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA, under ATCC No. 33015.

The enzyme induction can suitably be performed both with compounds which serve the microorganism as carbon source and energy source, such as p-xylene, m-xylene, p-cymene, m-cymene and toluene, and with compounds which do not serve the microorganism as carbon

source and energy source, such as monosubstituted and disubstituted methyl toluenes, ethyl toluenes and chlorotoluenes, benzyl alcohols and p-chlorobenzaldehyde, which have already been described as enzyme inducers for the degradation of aromatic hydrocarbons [Abril, M.-A., et al., J. Bacteriol., Vol. 171, (1989), pp. 6782 to 6789].

Preferably the enzyme induction is performed with p-xylene, m-xylene, 2-chlorotoluene or 2-bromotoluene.

The compounds utilized for induction can either be present during the reaction of the substrate or the supply thereof can be stopped before the reaction of the substrate. The inducer concentration is usually selected so that it is lower than the minimal inhibitory concentration of the enzymes responsible for the reaction. Depending on the embodiment of the process, the addition of the compounds used for the induction is preferably stopped during the reaction of the substrate, either by stopping the feeding or by centrifuging off the cells.

The strains used in the invention usually grow with p-xylene, m-xylene, p-cymene, m-cymene or toluene as the sole carbon source and energy source in a mineral medium [Kulla et al., Arch. Microbiol., 135, (1983), pp. 1 to 7] or in a complex medium ("Nutrient Broth Nr. 2", Oxoid Ltd., G.B.) or in a minimal medium, the composition of which is indicated in the following Table 3. The growth substrate was fed to the medium in gaseous form according to the data of Claus and Walker [J. Gen. Microbiol., 36, (1964), pp. 107 to 122], and the gassing rate was 0.5 V/min.

Before the substrate addition, the cells are cultured to an optical density of 1 to 200 at 650 nm in the culture medium, preferably up to an optical density of 5 to 100 at 650 nm.

The reaction can suitably take place either with a single or continuous substrate addition so that the substrate concentration in the culture medium does not exceed 20 percent (w/v). Preferably the substrate addition takes place so that the substrate concentration in the culture medium does not exceed 5 percent (w/v). Depending on the embodiment, the substrate addition can also take place at the same time as the enzyme inducer, for example, by a mixture of enzyme inducer and substrate being used.

The reaction is suitably performed in a pH range of 4 to 11, preferably from 6 to 10. Suitably the reaction is usually performed at a temperature of 15° to 0°C., preferably at a temperature of 25° to 40°C. The reaction is usually completed within a period of 1 to 24 hours.

As substrates for the reaction, methylated aromatic 5-member ring heterocycles which contain one or more heteroatoms from the series of oxygen, nitrogen and sulfur may advantageously be used, such as methylated thiophenes, methylated furans, methylated pyrroles, methylated thiazoles, methylated pyrazoles or methylated imidazoles, which are unsubstituted on the carbon atom adjacent to the methyl group to be oxidized. Preferably methylated furans, methylated thiophenes, methylated pyrroles and methylated thiazoles are used. 3,5-Dimethylpyrazole, 5-methylthiazole, 4-methylthiazole, 2,5-dimethylthiophene, 2-methylthiophene, 3-methylthiophene, 2,5-dimethylfuran and 2,5-dimethylpyrrole are especially suitable for use as the 5-member ring heterocycle.

The reaction can also be performed with aromatic methylated 6-member ring heterocycles with one or more nitrogen atoms as heteroatom, such as methylated pyridines, methylated pyrimidines, methylated pyrazines or methylated pyridazines, which are unsubstitued on the

carbon atoms adjacent to the methyl groups to be oxidized. Preferably methylated pyridines, methylated pyrazines and methylated pyrimidines, are used such as 2-methylpyridine, 3-methylpyridine, 4-methylpyridine, 2,5-dimethylpyridine, 2,4-dimethylpyridine, 6-chloro-3-methylpyridine, 2-chloro-3-ethyl-6-methylpyridine, 4,6-dimethylpyrimidine, 2-methylpyrazine, 2,5-methylpyrazine, 2-6-dimethylpyrazine, 2,3,5-trimethylpyrazine and 2-chloro-3,6-dimethylpyrazine.

A preferred embodiment of the process is depicted in Fig. 1. According to this embodiment, the enzyme inducer and/or the methylated heterocycle can be fed as substrate into a bioreactor (2), and the amount of feed is regulated by the concentration of the enzyme inducer in exhaust air (3) or the bioreactor (2). Preferably the concentration of the enzyme inducer in the exhaust air (3) is measured with a measuring device (4). The measuring device for the enzyme inducer concentration may be, for example, a photometer for ultraviolet light, a gas chromatograph or a gas chromatograph coupled with a mass spectrometer. This measuring device (4) is suitably coupled with a control device (5), which regulates the amount of feed of the enzyme inducer and/or of the methylated heterocycle by a pump (1), by which the feed of the enzyme inducer and/or of the methylated heterocycle to the bioreactor 2 takes place. The feeding of the enzyme inducer and/or of the methylated heterocycle is suitably regulated by this coupling. Preferably the concentration of the enzyme inducer in the exhaust air (3) is kept constant by this regulation. The regulation suitably takes place so that the concentration of the enzyme inducer in the exhaust air (3) is between 0.001 and 10 mmol/l of exhaust air, preferably between 0.01 and 3 mmol/l of exhaust air. Preferably the enzyme inducer and/or the methylated heterocycle is fed as substrate in the form of a mixture into the bioreactor

(2). The ratio of the enzyme inducer to substrate is suitably between 5:1 and 3:1. According to this embodiment, the enzyme inducer and/or the methylated heterocycle is suitably fed to the bioreactor (2) by means of the incoming air.

Fig. 2 shows the results of the biotransformation in the preferred embodiment.

After the reaction, the corresponding acids can be isolated in a known way, for example by extraction with organic solvent or, if the alkali salt of the heterocyclic carboxylic acid is formed, by concentration by evaporation of the cell-free culture medium. The alkali salt of the heterocyclic carboxylic acid can be formed, for example, by addition of an alkali hydroxide for adjustment of the pH of the culture medium.

Thus, according to the invention a simple, one-step microbiological process for the oxidation of methyl groups in aromatic 5- or 6-member ring heterocycles is afforded with which the corresponding acids are isolated in good yield and purity. Another advantage of this process consists in the fact that the aromatic heterocycle is not cleaved off and the reaction rate does not depend on the amount of the enzyme inducer.

The following Examples illustrate the invention.

Example 1

5-Methyl-2-pyrazine carboxylic acid

Pseudomonas putida ATCC No. 33015 was cultured in a complex medium (100 ml) "Nutrient Broth Nr. 2" (Oxoid Ltd., England) in a fermenter at pH 7.0 and at a temperature of 30°C. The enzyme inducer p-xylene was fed in gaseous form according to the data of Claus and Walker [J. Gen. Microbiol., 36, (1964), pp. 107 to 122] up to a concentration of 1 mmol/liter. Then the cells were washed twice with mineral medium [Kulla et al., Arch. Microbiol., 135, (1983), pp. 1 to 7] and an optical density of 10 at 650 nm in 100 ml of mineral medium was

set. 1 mmol of 2,5-dimethylpyrazine, which corresponds to a substrate concentration of 0.108 percent (w/v) in 100 ml, was added to this cell suspension. After an incubation period of 4 hours at 30°C there was obtained, in the absence of the enzyme inducer, 0.9 mmol of 5-methyl-2-pyrazine carboxylic acid corresponding to a yield of 90 percent, based on the 2,5-dimethylpyrazine used.

Example 2

Analogous to the procedure of Example 1, various additional compounds were also used as enzyme inducer, and the results are summarized in Table 1 with the corresponding concentration.

Table 1

Enzyme inducer	Concentration moles in 100 ml of cells (mmol)	Substrat amount [1 mmol in 100 ml of cells corr. 0.108% (w/v)]	Percent Yield of 5-methyl-2-pyrazine carboxylic acid
x-xylene	0.1	2,5-dimethyl pyrazine	90
m-xyléne	0.1	2,5-dimethyl pyrazine	90
2-chloro-toluene	0.1	2,5-dimethyl pyrazine	90
2-bromo-toluene	0.1	2,5-dimethyl pyrazine	90

Example 3

5-Methyl-2-pyrazine carboxylic acid

Pseudomonas putida ATCC No. 33015 was cultured according to Example 1 but in mineral medium Kulla et al., Arch. Microbiol., 135, (1983), pp. 1 to 7] with p-xylene as the sole carbon source and energy source. 1 mmol of 2,5-dimethylpyrazine, which corresponds to a concentration of 0.108 percent (w/v), was added

to the cell suspension (100 ml) with an optical density of 10. The p-xylene addition was stopped during the reaction of the substrate. Under these conditions, 1 mmol of 2,5-dimethylpyrazine was converted over a period of 4 hours to 0.9 mmol of 5-methyl-2-pyrazine carboxylic acid, corresponding to a yield of 90 percent, based on the 2,5-dimethylpyrazine used.

Example 4

Pseudomonas putida DSM 5709 was cultured analogously to Example 3, but with p-cymene as sole carbon source and energy source. After a period of 16 hours, 0.5 mmol of 5-methyl-2-pyrazine carboxylic acid was obtained, corresponding to a yield of 50 percent, based on the 2,5-dimethylpyrazine used.

Examples 5 to 28

Examples 5 to 28 were performed following the procedure of Example 3 with an amount of 1 mmol of substrate per 100 ml of cell suspension and the results are summarized in Table 2.

Table 2

Ex.	Substrate	Concentration of substrate in % (w/v) in the culture medium	Reaction Time in hours	End Product	Yield in %
5	2-methyl pyrazine	0.049	16	2-pyrazine carboxylic acid	30

6	2,5-dimethyl-pyrazine	0.108	4	2-methyl-5-pyrazine carboxylic acid	90
7	2,6-dimethyl-pyrazine	1.108	4	2-methyl-6-pyrazine carboxylic acid	90
8	2,3,5-trimethyl-pyrazine	0.122	16	2,3-dimethyl-5-pyrazine carboxylic acid	50
9	2-chloro-3,6-dimethyl-pyrazine	0.142	16	2-chloro-3 methyl-6-pyrazine carboxylic acid	90
10	2-methyl-pyridine	0.093	16	2-pyridine carboxylic acid	90
11	3-methyl-pyridine	0.093	16	3-pyridine carboxylic acid	50
12	4-methyl-pyridine	0.093	16	4-pyridine carboxylic acid	30
13	2,6-dimethyl-pyridine	0.107	16	6-methyl-2-pyridine carboxylic acid	80
14	2,5-dimethyl-pyridine	0.107	16	5-methyl-2-pyridine carboxylic acid	40
15	2,4-dimethyl-pyridine	0.107	16	4-methyl-2-pyridine carboxylic acid	40
16	3,5-dimethyl-pyridine	0.107	16	5-methyl-3-pyridine carboxylic acid	40

17	6-chloro-2-methyl-pyridine	0.128	16	6-chloro-2-pyridine carboxylic acid	90
18	6-chloro 3-methyl-pyridine	0.128	16	6-chloro-3-pyridine carboxylic acid	90
19	2-chloro-3-ethyl-6-methyl-pyridine	0.157	16	2-chloro-3-ethyl-6-pyridine carboxylic acid	10
20	4,6-dimethyl-pyrimidine	0.108	16	6-methyl-4-pyrimidine carboxylic acid	20
21	3,5-dimethyl-pyrazole	0.096	16	5-methyl-3-pyrazole carboxylic acid	80
22	5-methyl-thiazole	0.099	16	5-thiazole carboxylic acid	80
23	4-methyl-thiazole	0.099	16	4-thiazole carboxylic acid	80
24	2,5-dimethyl-thiophene	0.112	16	5-methyl-2-thiophene carboxylic acid	90
25	2-methyl-thiophene	0.098	16	2-thiophene carboxylic acid	90
26	3-methyl-thiophene	0.098	16	3-thiophene carboxylic acid	90
27	2,5-dimethyl-furan	0.096	16	5-methyl-2-furan carboxylic acid	40

28	2,5-dimethyl-pyrrole	0.095	16	5-methyl-2-pyrrole carboxylic acid	40	

Example 29

Production of 5-methyl-2-pyrazine carboxylic acid

Pseudomonas putida ATCC No. 33015 was cultured in minimal médium, the composition of which is indicated in Table 3 below, in a 20-liter bioreactor (2) at a working volume of 15 liters. The temperature was 30°C; the pH was kept constant at 7.0 by the addition of potassium hydroxide. The gassing rate was 20 liters per minute. A mixture of 4 parts (v/v) of p-xylene and 1 part of 2,5-dimethylpyrazine served as the growth substrate. Feeding of this mixture into the bioreactor (2) took place by means of the pump (1). The addition of the growth substrate was coupled to a control (5) by a xylene measurement (4) in bioreactor exhaust air (3). Thus, by means of the control (5), the xylene concentration in the exhaust air (3) was kept constant at a value of 0.2 mmol/l. The biotransformation was stopped only when no more growth could be detected. Fig. 2 shows the results of such a biotransformation. Curve A of Fig. 2 represents the optical density at 650 nm. Curve B of Fig. 2 represents the concentration in g/l of 2,5-dimethylpyrazine. Curve C of Fig. 2 represents the concentration in g/l of the potassium salt of 5-methyl-2-pyrazine carboxylic acid. With this technology, 5-methyl-2-pyrazine carboxylic concentrations of 38 g/k

were obtained.

Table 3

Medium composition:

Ingredients	Amounts
$MgCl_2 \cdot 6H_2O$	0.8 g/l
$CaCl_2$	0.16 g/l
$(NH_4)SO_4$	2 g/l
NH_4Cl	5 g/l
Na_2SO_4	0.25 g/l
KH_2SO_4	0.4 g/l
Na_2HPO_4	0.9 g/l
Trace elements	1 ml/l
FeEDTA	15 ml/l

Composition of the trace element solution:

Ingredients	Amounts
KOH	15 g/l
$EDTANa_2 \cdot 2H_2O$	10 g/l
$ZnSO_4 \cdot 7H_2O$	9 g/l
$MnCl_2 \cdot 4H_2O$	4 g/l
H_3BO_3	2.7 g/l
$CoCl_2 \cdot 6H_2O$	1.8 g/l
$CuCl_2 \cdot 2H_2O$	1.5 g/l
$NiCl_2 \cdot 6H_2O$	0.18 g/l
$Na_2MoO_4 \cdot 2H_2O$	0.2 g/l

Composition of FeEDTA:

Ingredients	Amounts
$EDTANa_2 \cdot 2H_2O$	5 g/l
$FeSO_4 \cdot 7H_2O$	2 g/l

Comparison Examples

(A) Pseudomonas putida ATCC 33015

The biotransformation of 3-methylpyridine with Pseudomonas putida ATCC No. 33015 was performed according to Example 3 with 1 mmol of 3-methylpyridine. After an incubation period of 8 hours, 0.25 mmol of nicotinic acid was obtained, corresponding to a yield of 25 percent, based on the 3-methylpyridine used.

(B) Rhodoccocus rhodochrous or Nocardia

Rhodoccocus rhodochrous DSM 43002 (ATCC No. 19149) was cultured in a mineral medium according to Example 3, with 0.4 percent dodecane as the carbon source and the energy source and then washed in the same mineral medium, but without dodecane. The cell suspensions (100 ml) with an optical density at 650 nm of 10 were mixed in three separate flasks with the following compounds:

(a) 1 mmol of 3-methylpyridine, (b) 1 mmol of 3-methylpyridine and 0.055 mmol of dodecane, and (c) 1 mmol of 3-methylpyridine and 5.5 mmol of dodecane.

After 8 hours of incubation time at 30°C, nicotinic acid could not be detected in any of the batches.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A microbiological process for the oxidation of a methyl group in an aromatic 5- or 6-member ring heterocycle to form the corresponding carboxylic acid, which heterocycle is unsubstituted on the carbon atom adjacent to the methyl group to be oxidized, wherein the methylated heterocycle is used as the substrate for the reaction and the reaction is performed by microorganisms of the genus Pseudomonas utilizing toluene, xylene or cymene, enzymes of the microorganisms having been previously induced.

2. A process as claimed in claim 1, wherein the enzyme induction is performed either with a compound which serves the microorganism as the carbon source and the energy source, or with a compound which does not serve the microorganism as the carbon source and the energy source.

3. A process as claimed in claim 2, wherein the reaction is performed by microorganisms of the species Pseudomonas putida utilizing toluene, xylene or cymene.

4. A process as claimed in claim 1 or 3, wherein the reaction is performed with the xylene-utilizing microorganism strain Pseudomonas putida ATCC No. 33015 or an effective mutant of said strain.

5. A process as claimed in claim 1 or 3, wherein the reaction is performed with the cymene-utilizing microorganism strain Pseudomonas putida DSM 5709 or an effective mutant of said strain.

6. A process as claimed in claim 1, 2 or 3, wherein the reaction takes place either with a single or continuous substrate addition so that the substrate concentration in the culture medium does not exceed 20 percent (w/v).

7. A process as claimed in claim 1, 2 or 3, wherein the reaction is performed at a pH of 4 to 11.

8. A process as claimed in claim 1, 2 or 3, wherein the reaction is performed at a temperature of 15° to 50°C.

9. A process as claimed in claim 1, 2 or 3, wherein the reaction is performed with a methylated aromatic 5-member ring heterocycle, as the substrate, which contains one or more heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur.

10. A process as claimed in claim 1, 2 or 3, wherein the reaction is performed with a methylated thiophene, methylated furan, methylated pyrrole, methylated thiazole, methylated pyrazole or methylated imidazole as the substrate.

11. A process as claimed in claim 1, 2 or 3, wherein the reaction is performed with a methylated aromatic 6-member ring heterocycle, as the substrate, which contains one or more nitrogen atoms as the heteroatom.

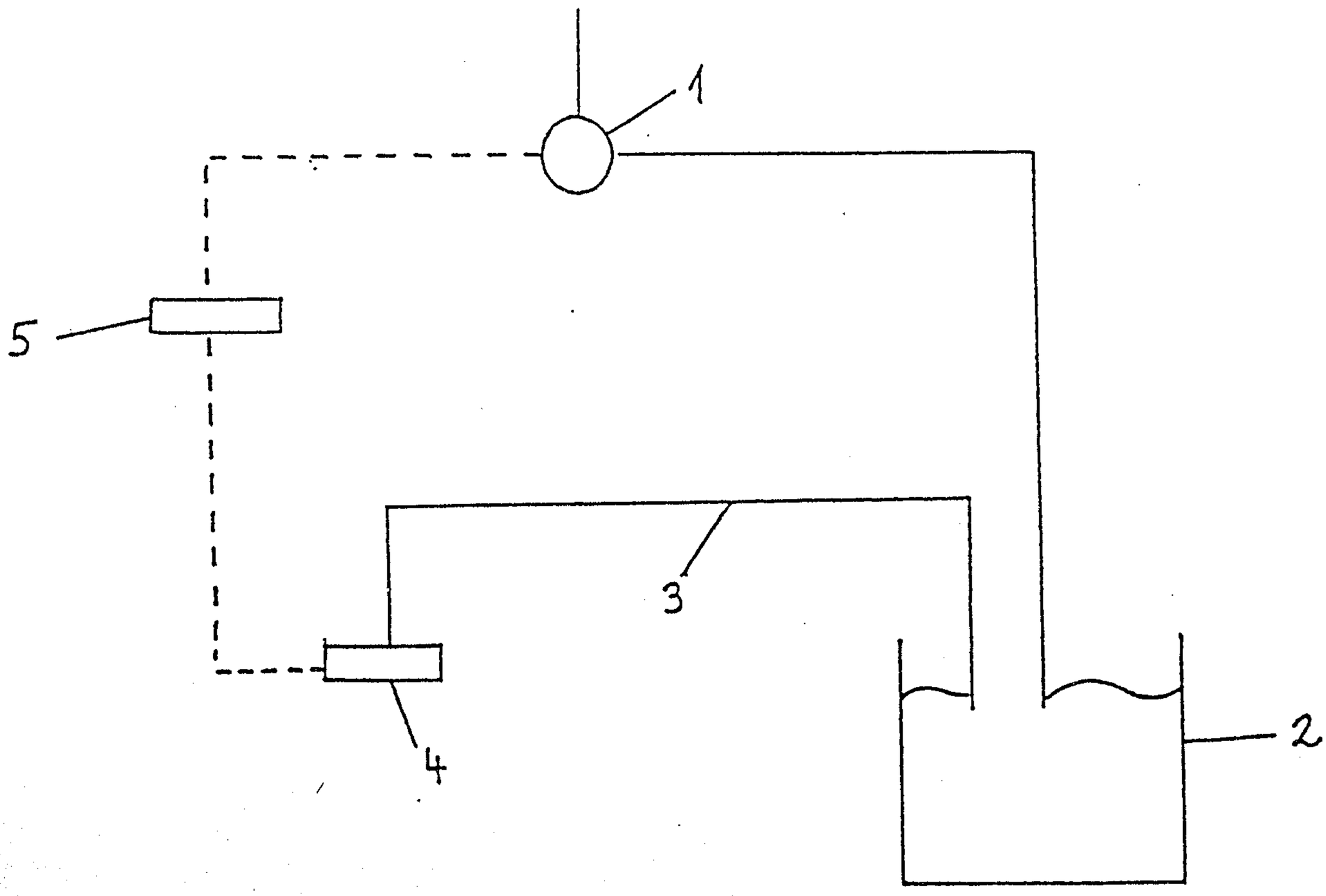
12. A process as claimed in claim 1, 2 or 3, wherein the reaction is performed with a methylated pyridine, methylated pyrimidine, methylated pyrazine or methylated pyridazine as the substrate.

13. A process as claimed in claim 1, wherein the enzyme inducer and/or the methylated heterocycle is fed as the substrate into bioreactor, and the amount of the feed is regulated by the concentration of the enzyme inducer in the exhaust air of the bioreactor.

14. A process as claimed in claim 13, wherein the concentration of the enzyme inducer is measured in the exhaust air with a measuring device and the amount of feed of the enzyme inducer and/or of the methylated heterocycle is regulated by a control, which is coupled to the measuring device.

15. A process according to claim 13 or 14, wherein the regulation takes place so that the concentration of the enzyme inducer in the exhaust air is kept constant and is between 0.001 and 10 mmol/1 of exhaust air.

Fig.1

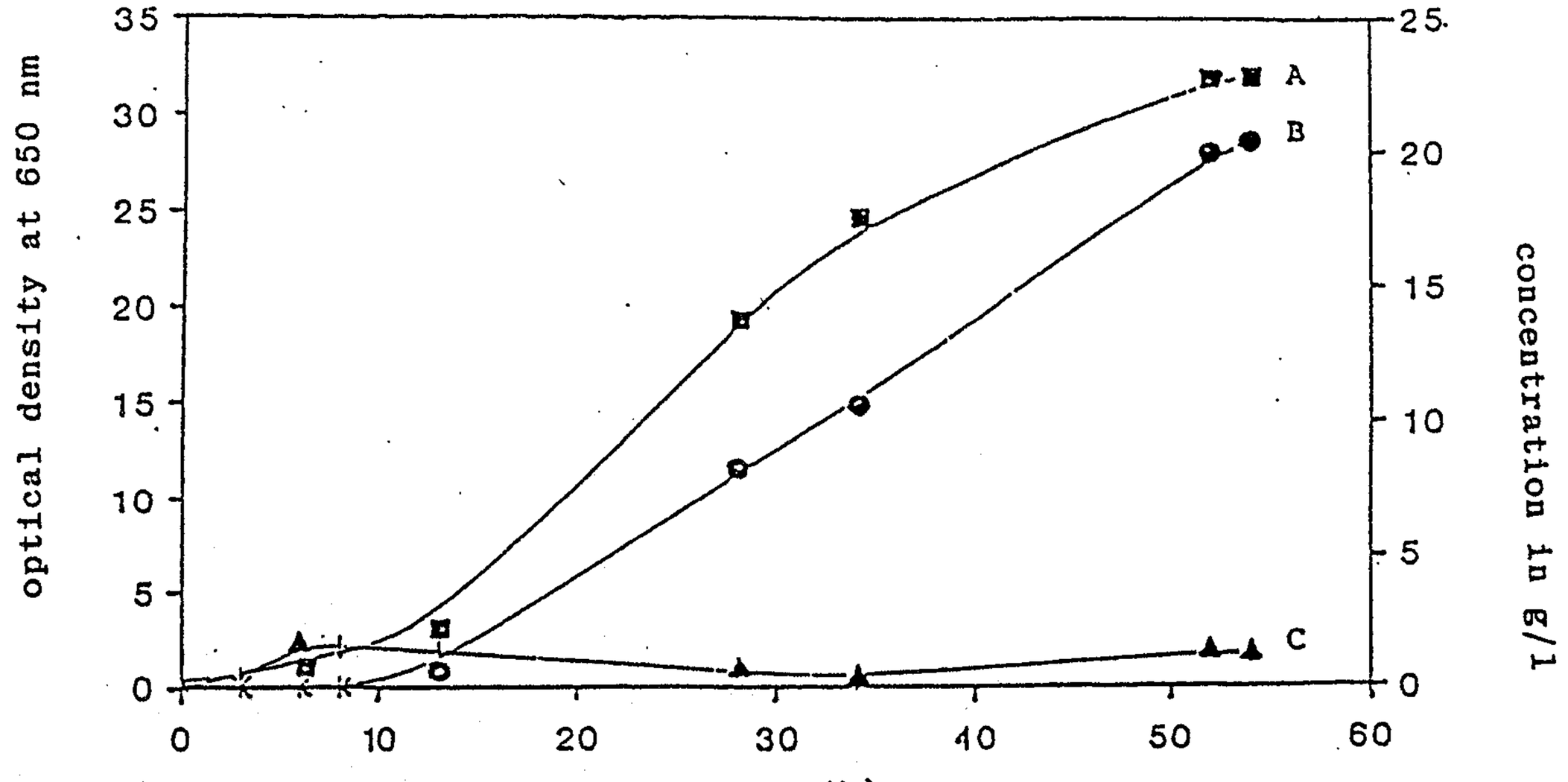


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MICROBIAL FORMATION OF 5-METHYL-2-PYRAZINE CARBOXYLIC ACID

Fig. 2



A ■ Optical density

B ▲ 2,5-Dimethylpyrazine (g/l)

C ● Potassium salt of 5-methyl-2-pyrazine carboxylic acid (g/l)

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